

# **The Development and Implementation of Strategies to Genetically Reduce Methane Emissions in Australian Dairy Cattle**

Submitted by

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## List of Abbreviations

ABV	Australian breeding value
AUD\$	Australian dollar
BPI	Balance Performance Index
$\Delta$ BW	Change in bodyweight
BCS	Body condition score
CAD\$	Canadian dollar
CO <sub>2</sub>	Carbon Dioxide
CO <sub>2</sub> -eq	Carbon Dioxide Equivalents
DIM	Days in milk
DMI	Dry matter intake
DNA	Deoxyribonucleic acid
EBV	Estimated breeding value
ECM	Energy corrected milk
FTIR	Fourier-transform infrared
GEBV	Genomic estimated breeding value
GHG	Greenhouse gas
GWAS	Genome-wide association
GWP	Global warming potential
h <sup>2</sup>	Heritability
H <sub>2</sub>	Hydrogen
H <sub>2</sub> O	Water
HWI	Health weighted index
MBW	Metabolic bodyweight
MIR	Mid-infrared
n	Sample size
N <sub>2</sub> O	Nitrous oxide
r <sup>2</sup>	Reliability
R <sup>2</sup>	Coefficient of determination
RFI	Residual feed intake
RMP	Residual methane production

SCC	Somatic cell count
SF <sub>6</sub>	Sulfur hexafluoride
USD\$	United States dollar
VFA	Volatile fatty acids

## Abstract

In agriculture, sustainability has a complex definition that considers economic profitability, social responsibility, and environmental stewardship. This robust definition of sustainability involves every aspect of the commodity chain and affects the entire dairy production system from the cow and the farmers to the consumer and the larger societal perception. Reducing methane and overall emissions is a component of the breeding objective required to create a sustainable dairy animal in the future. However, acquiring methane data on many cows to estimate genomic prediction equations is challenging, as methane is expensive and laborious to measure. The aim of this thesis was to investigate and develop a genetic-based mitigation tool to reduce emissions in dairy cattle, focusing on methane emissions. Firstly, nine definitions of residual methane production (**RMP**) were compared, including a genetic and phenotypic regression of methane production on a combination of dry matter intake (**DMI**) and energy corrected milk (**ECM**). RMP candidate traits had low to moderate heritability estimates (0.10 to 0.21) and all definitions of RMP had high genetic correlations with one another ( $>0.79$ ) and other methane candidate traits ( $>0.59$ ). A methane trait phenotypically corrected for energy corrected milk was computationally less complex and therefore potentially preferable. An alternative approach was to reduce emissions through genetic selection to lower replacement rates and increase efficiency of production. Therefore, coefficients of current index traits on emissions produced per animal due to genetic improvement were estimated and compared. The traits that had an impact on emissions include milk, fat, protein, fertility, survival, and feed efficiency. These coefficients were used to develop sub-indexes to predict emission, some included the previously estimate RMP trait. Finally, the possible options to reduce greenhouse gas emissions in the Australian dairy industry were investigated by: 1) including the GHG sub-index in the national breeding program, and 2) estimating the economic and environmental impact of implementation of the subsequent indexes. A total of 12 possible selection indexes were compared with three scenarios depending on availability and efficacy of a direct methane trait breeding value prediction and four different carbon prices. By implementing a GHG sub-index in the national breeding program, we can achieve up to a 7.9% decrease in RMP and nine times the reduction in gross emissions in 10 years compared to the current breeding program, with little to no cost to farmers. Therefore, offering a mitigation strategy that will be effective at reducing emissions with little compromise to profit.

## **Statement of Authorship**

Except where reference is made in the text of the thesis, this thesis contains no material published elsewhere or extracted in whole or in part from a thesis accepted for the award of any other degree or diploma. No other person's work has been used without due acknowledgment in the main text of the thesis.

This thesis has not been submitted for the award of any degree or diploma in any other tertiary institution. The extent and nature of contributions by co-authors are specified in Appendix 1.

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## Acknowledgements

In 1955, Lawrence C. Mindham sat in the living room his quaint English home and flipped a coin - it landed on tails. He packed up his bags and moved to Canada where he met his beautiful wife, had three children and eight grandchildren – one of which was me. If the coin had landed on tails, my grandfather would have been packing his swimsuit rather than his snowsuit and headed to his alternative destination of Australia. It is only fitting that my academic career brought me to what could have been, and now is, my second home.

During my time in Australia, I have met so many wonderful people and created friendships that will last a lifetime. Even through the events of COVID, we've shared experiences and made endless memories, with so many more still to come. From my advisors to the barista at my favorite coffee shop, thank you for the endless support you have given me. Although, none of this would have been possible without the generous financial support of La Trobe University and DairyBio, jointly funded by Agriculture Victoria, Dairy Australia and the Gardiner Foundation, of which I am incredibly grateful.

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To the Computation Biology team, and my friends and colleagues at Agriculture Victoria Research, thank you for allowing me to be a part of such an exceptional team. My sincere thanks to each of you for your contributions – in one way or another – to my thesis. I look forward to seeing how you continue to drive innovation and build the foundation of our industry in the future.

Thank you to all my friends in Australia - life would certainly not have been the same without you. To Bryce, Alex and the Yaps, thank you for making sure I had a life outside of academia and for helping me make every wild idea into an unforgettable memory. Tim, thanks for helping me realize that not every idea is a good idea and that more work doesn't always mean better results – in life and work.

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## **Preface**

This thesis consists of six chapters. Chapter 1 is a general introduction which provides an overview of the research topic and outlines the main objectives of each thesis chapter. Chapters 2 to 5 are original research undertaken as part of this project, which have been published in peer-reviewed scientific journals. These research chapters follow the formatting style of the respective journal specifications. For each of the research chapters, I held the primary responsibility and role of designing the study, cleaning and analysing all data, interpreting the results, writing of each manuscript, and obtaining additional funding opportunities. My supervisors and co-authors provided support and guidance on the design, analysis, interpretation of the results, and contributed to the writing of manuscripts. Chapter 6 is of my own thinking and a general discussion of the research, and considerations on top of those described in Chapter 5 that may be used for the successful implementation of a genetic tool to reduce GHG emissions and improve the long-term sustainability of the Australian dairy industry. Finally, peer-reviewed conference materials as well as competition submission materials for the Australian Young Dairy Scientist of the Year, to which I was awarded in 2019, are provided in Appendix 1 to 3. Formatting styles of the conference materials and competition submission materials follow the guidelines from the respective conference proceedings.

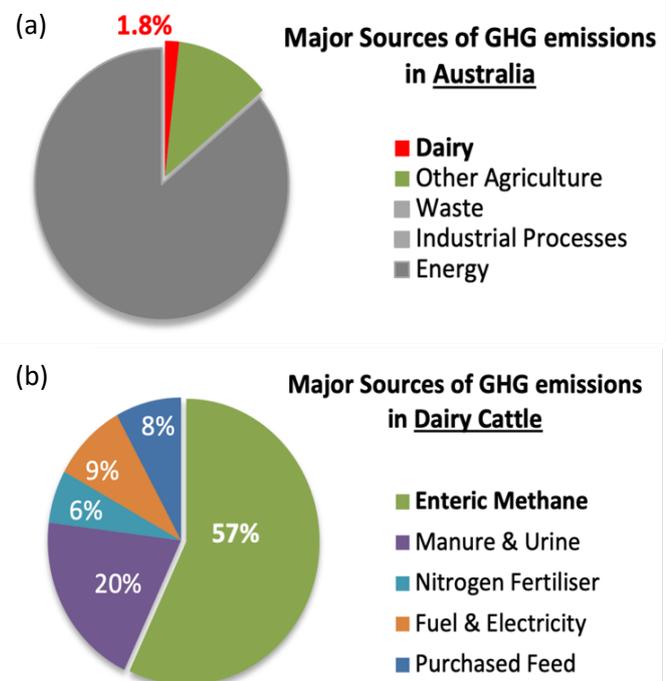
# **Chapter 1:**

## **General Introduction**

## 1.1 Global warming

In 2017, human-induced global warming reached 1°C above pre-industrial (1850-1900) levels, increasing at an alarming rate of 0.2°C per decade (IPPC, 2021). At this rate, the detrimental effects of climate change are projected to be catastrophic with more severe storms, longer droughts, hotter temperatures, and rising sea levels (NASA, 2021). Therefore, adaptations need to be made by all industries to reduce overall emissions and ensure that global warming remains below the critical level of 1.5°C. The Paris Agreement is a legally binding international treaty with the collective goal of reducing greenhouse gas (GHG) emissions to limit global warming to 1.5°C by 2030 (Paris Agreement, 2016). In 2016, Australia became one of the 195 signatories and committed to reduce its GHG emissions by 26 to 28% of 2005 levels by 2030 (Paris Agreement, 2016). This target relies largely on a reduction in the three major atmospheric GHGs: carbon dioxide (CO<sub>2</sub>), methane, and nitrous oxide (N<sub>2</sub>O), which account for 81%, 10% and 7% of the global GHG inventory, respectively (epa.gov).

Australia's 2020 national emissions inventory was 499.0 Mt CO<sub>2</sub>-e, with the largest emitting sectors being electricity (33.6%) and stationary energy (20.4%). Agriculture contributed 14% to Australia's national emissions inventory (Figure 1), of which 12.5% were due to the dairy industry. Although the dairy industry only contributes 1.8% to Australia's total national emission inventory,



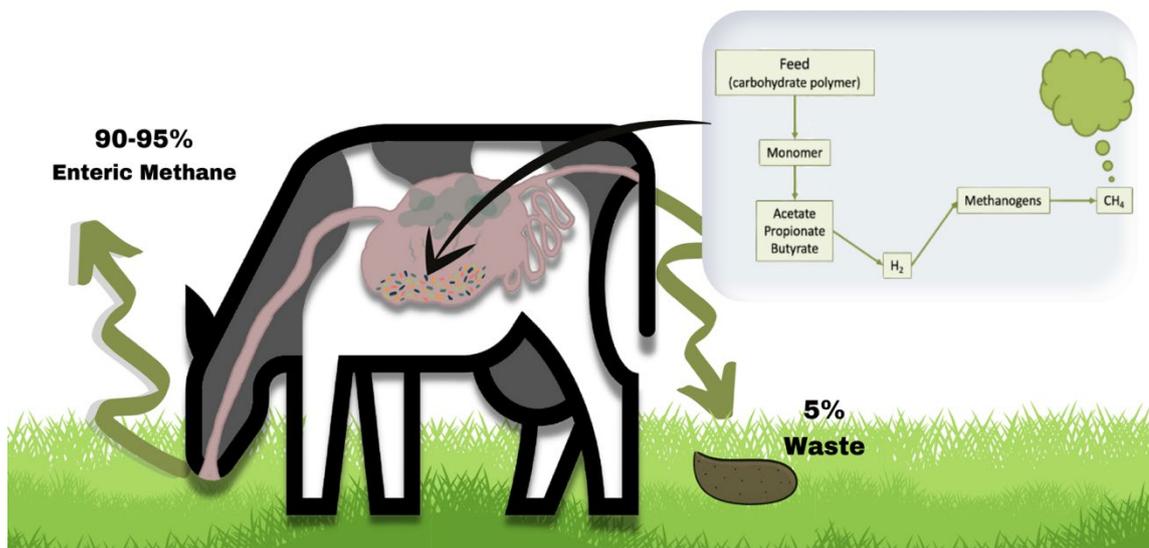
**Figure 1:** Major emissions by sector (a) and sources of greenhouse gas (GHG) in Australian dairy cattle (b) (Christie et al., 2016).

approximately 57% of the emissions generated by the dairy industry are due to enteric methane (Christie et al., 2016).

## 1.2 Methane emissions in relation to dairy cattle

Enteric methane is a volatile GHG that is commonly associated with domestic livestock production but is also generated through other human activities (including natural gas and petroleum production, landfill waste decomposition, and coal mining) and natural sources (such as wetlands). However, the most significant anthropogenic methane source in the global methane budget is agriculture due to enteric fermentation and manure management (Sauniois et al., 2019). Although the atmospheric lifespan of methane is much shorter than CO<sub>2</sub>, methane is a more potent GHG due to its ability to efficiently trap radiation, with a global warming potential (GWP) of 28 compared to CO<sub>2</sub> which has a GWP of 1 (Myhre et al., 2013).

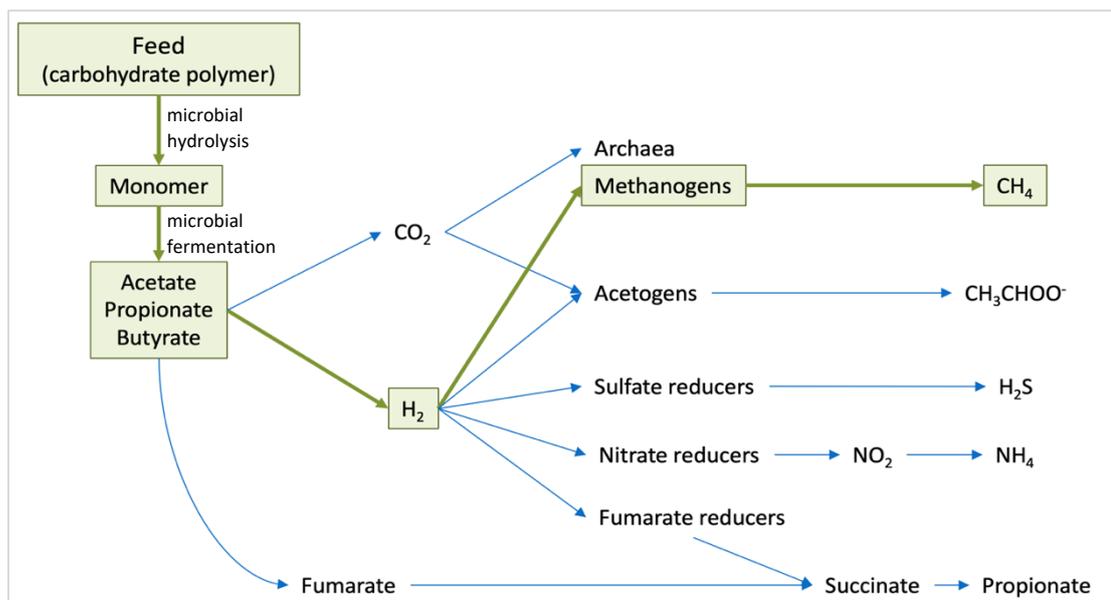
In animal production, enteric methane production has been associated with the practice of raising and maintaining ruminant production animals (Cottle et al., 2011). Ruminants are a division of animals, including cattle, sheep and goats, that survive on



**Figure 2:** In cattle, methane is primarily generated as a by-product of feed fermentation. Through this process, 90-95% of the methane a cow produces is enteric methane and generated by microorganism called methanogens which capture excess hydrogen that was released during the fermentation process (Broucek, 2014).

plant-based diets through the process of foregut fermentation or rumination. This process allows the animal to convert cellulose fiber into energy which is accessible for maintenance, growth, and production. It also allows the animal to convert human indigestible feeds into nutrient dense, widely accessible human food sources. Through the process of fermentation, hydrogen is created and subsequently converted to enteric methane. Although not all methane produced by ruminant animals is enteric methane, this process accounts for approximately 90 - 95% of the methane produced by cattle (Figure 2; Broucek, 2014).

The production of enteric methane is a small component of the complex biochemical digestive process of the cow (Figure 3). In the reticulorumen and hindgut of the cow, hydrolyzed 5- and 6-carbon sugars are fermented to volatile fatty acids through a multi-step process which produces metabolic hydrogen that is then converted to the more stable  $H_2$ . The accumulation of dissolved  $H_2$  alters the pH of the rumen and inhibits fermentation through negative feedback mechanisms, which prevent the re-oxidization of reduced co-factors and inhibits the production of volatile fatty acids. Therefore, it is vital that the excess  $H_2$  is removed to ensure that proper carbohydrate degradation, rate of microbial growth, and microbial protein synthesis are maintained



**Figure 3:** Biochemical processes and pathways of feed fermentation, where 6-12% of the energy in feed is used to generate enteric methane (Appuhamy et al., 2016).

(Knapp et al 2014). Microorganisms called methanogens help to maintain rumen pH and healthy digestion by converting the H<sub>2</sub> and CO<sub>2</sub> to methane and water through the process methanogenesis (Hungate, 1966; Czerkawski, 1986; Moss et al., 2000). Therefore, some production of enteric methane is vital to trap and expel excess hydrogen and maintain the healthy digestive system of the animal. However, the variation in enteric methane that exists between animals suggests that producing methane may be a source of on-farm inefficiency with up to 6-12% of feed energy being driven towards methane production (Johnson et al. 1993; Okine et al. 2004; Appuhamy et al., 2016).

### **1.3 The Australian dairy population**

The Australian national dairy herd is characteristically variable in terms of herd size, calving system, breed, production level, milking structure, and feeding management system. There are currently 1.41 million milking cows and 5213 dairy farms in Australia. The average herd consists of 279 cows; however, herd size can range between 30 to 5000 cows (Dairy Australia, 2019). The primary calving system practiced is seasonal calving, although both year-round and split-year calving systems are gaining popularity. There are a total of five feeding systems, with 35% of animals consuming pasture and the remaining receiving a diet ranging from concentrate added pastoral systems to complete total mixed ration. Twice a day milking is the most common milking protocol, but both once per day and three times per day milking protocols are also practiced. Dairy farms are located across the many Australian sub-climates, with the majority of farms located in the southeast of Australia. Within the population of cattle enrolled in routine milk testing programs, Holsteins are the most prominent breed (72%), however, Jerseys (15%), Aussie Reds (4%), and Jersey x Holstein crossbreds (5%), as well as Ilawarra, Brown Swiss, Guernsey and Ayrshire, are gaining in popularity (DataGene, 2020). The wide range of on-farm characteristics increases the complexity of developing a single national breeding objective that meets

the needs of each farmer, including developing an industry wide emission mitigation strategy.

#### **1.4 Mitigation Options**

To help Australia meet its GHG mitigation targets, the dairy industry has committed to reducing its emission intensity by 30% by 2030 (Dairy Australia, 2021). New Zealand has set a more aggressive target of reducing enteric methane from dairy cattle by 10% of 2017 levels by 2030 (DairyNZ, 2019). Other dairy industries have also aligned their industry goals to pursue a more net-neutral production system (European Dairy Association 2019; Dairy Farmers of Canada, 2020, DairyUK, 2021).

Many mitigation options have been suggested to reduce emissions and improve efficiency in the dairy cattle industry, which are thoroughly reviewed by Knapp et al. (2014). One of the most widely investigated reduction strategies is the use of feed additives and dietary manipulations. These include the use of high lipid feeds, such as brewer's grains, cold-pressed canola, grape marc, and wheat (Moate et al 2016); plant compounds and extracts, such as tannins (Grainger et al., 2009) and essential oils (Hart et al., 2019); and methane inhibitors, such as monensin (Callaway et al 2003) and seaweed (Vijn et al., 2020). These products reduce enteric methane by altering ruminal fermentation, directly inhibiting methanogens, or by removing excess hydrogen and limiting methanogenesis. Other options that require further investigation include probiotics, bacteriocins, archaeal viruses and vaccinations (Williams et al 2009). In addition to changes in daily management practices and increases in labor requirement, these options represent a substantial cost to the producer. Additional mitigation opportunities with low inputs at the farm level focus on animal breeding and genetics.

There are several strategies that could be incorporated into breeding programs to target a reduction in methane emissions and a mitigation strategy centered around genetic selection has marginal additional cost, or labour expense to the farmer. Additionally, genetic selection offers a permanent and cumulative solution to reduce

GHG emissions, with the reduction in emissions continually building on the previously made progress. However, there are challenges in obtaining the phenotypes required for genetic evaluations of environmental traits.

### **1.5 Measuring techniques**

Direct selection for reduced methane emissions is challenging as collecting phenotypes is difficult and expensive (Garnsworthy et al., 2019), resulting in a small sample size. The benefit and drawback of each measurement technique, including the respiration chambers, sulfur hexafluoride (SF<sub>6</sub>) method, Sniffers, GreenFeed, and laser analyzers, have been previously summarized (Lassey 2007; Storm et al., 2012; Garnsworthy et al 2019).

The gold standard method of measuring methane is by using respiration chambers (Grainger et al., 2007), which offers the most accurate and repeatable methane measurements. However, this method is labour intensive, expensive and time consuming as only one animal at a time can be measured. This limitation means that it's only possible to collect data on a small number of animals. In respiration chambers animals are also isolated from the herd resulting in high behavioural alterations. The SF<sub>6</sub> method utilizes a wearable device that allows more natural behaviours to be expressed by avoiding disruption to herd interconnections. It is less expensive and requires less labour meaning that more animals can be measured at a given time, while also having a high concordance to the chamber technique (0.8; Deighton et al 2014). GreenFeed is comparable in data volume and quality; however, both these methods can be moderately expensive and labour intensive depending on the configuration of the research or commercial farm facility (Hammond et al., 2015). The sniffer analyzer technique measures breath samples during milking or feeding and generates a higher number of observations as it is inexpensive and has a comparatively low labour requirement. This method has reasonable repeatability, requires minimal labour resources, and avoids any possible behavioural interruptions (Garnsworthy et al., 2012); however, costs can vary depending on the sniffer equipment used. Although the laser

method has a lower repeatability than the less portable methods, its hand-held capabilities and low cost make it a more convenient option with higher throughput capabilities (Chagunda et al., 2009).

These methods are used globally to build phenotypic methane reference populations; however, only small datasets have been recorded due to the limitations of each method. Therefore, combining datasets to build databases similar to those constructed for dry matter intake and large enough for genetic evaluations is likely to be the most effective approach (de Haas et al., 2015). The multitude of measurement techniques adds complexity when combining international datasets, as correlations between methods is typically much less than 1 (Ellis et al., 2007). This challenge may be overcome by greatly increasing dataset size through the use of proxy traits.

## **1.6 Predicted and proxy traits**

Various proxy trait definitions have been proposed to increase the number of methane phenotypes available in dairy cattle, as thoroughly reviewed by Negussie et al (2017). One of the first predictions of methane emissions was through mathematical equations based on relevant traits physiologically linked to methane production. Early prediction equations were first developed using production data, with more recent equations including more detailed records such as body weight and milk fatty acid composition (Dijkstra et al., 1992; Benchaar et al., 2003; Huhtanen et al., 2015). Several studies have been conducted to examine the integrated rumen activities and biochemical pathways activated during the process of converting feed material to methane with the aim of identifying possible indirect phenotypes of methane. This includes correlated traits with an indirect response in methane, such as feed intake and feeding behavior (Moraes et al., 2014), rumen pH (Moate et al., 2020), microbiome (Wallace et al., 2015), and volatile fatty acid profiles (Alemu et al., 2011). These phenotypes could potentially be measured at a much higher volume than some of the previously describes direct methane measurement methods.

Methane may also be predicted using mid-infrared (**MIR**) spectroscopy of milk samples collected during routine herd improvement testing, offering an inexpensive and relatively simple alternative to gold standard testing (Vanlierde et al., 2015). The use of MIR predicted phenotypes has been previously investigated and successfully incorporated in the genetic evaluation for index traits, such as fat and protein (Soyeurt et al., 2011). Studies have examined the ability of MIR to predict methane in research populations (Vanlierde et al. 2018) with variable accuracies. In Australia, a previous methane MIR calibration equation with a  $R^2$  of 0.51 was derived using methane measured with the  $SF_6$  technique on research animals fed ad libitum alfalfa cubes over a 5-day period (Wang et al. 2017). However, no MIR methane prediction equation is currently implemented in genomic prediction of dairy cattle due to the herd-by-herd validation required (Wang et al., 2019).

Approximately 46% of Australian dairy cows are currently involved in routine milk testing programs, with the rate steadily increasing. Therefore, identifying an indirect methane phenotype that may be simply, and fairly accurately, measured on a large number of animals would dramatically increase the data available for routine genetic evaluations of this challenging and expensive to measure trait.

## **1.7 Genetic selection of novel traits**

The primary objective of animal breeding is to achieve genetic improvement by choosing animals with the highest genetic merit to become parents of the next generation. At its very simplest form (i.e. ignoring interactions), an animal's performance may be broken down into genetic and environmental factors:

$$P_i = G_i + E_i$$

where  $P_i$  is the phenotypic performance of the  $i^{\text{th}}$  animal,  $G_i$  is genetic effect of the  $i^{\text{th}}$  animal, and  $E_i$  is the environmental effect of the  $i^{\text{th}}$  animal. Environmental effects may include many factors such as birth year, calving season, parity, as well as residual effects. While the genetic component is often assumed to be only due to the additive

genetic effect, as these are the effects that are transmitted from one generation to the next.

Dairy cattle breeding programs have traditionally been driven by traits of economic importance, either through generating profit or by reducing expenses. Traits included in this type of breeding scheme have historically included milk and its components (fat and protein) which generate profit, and more recently have expanded to traits related to improved health, fertility and longevity which reduce expenses (Miglior et al., 2017). Breeding programs require two primary components: 1) a reference population composed of a substantial number of animals with phenotypes that accurately represents the general population and 2) a pedigree that accurately identifies previous breeding pairs. With this information, statistical models could be applied to estimate breeding values that rank animals based on genetic potential. In 1997, Nejati-Javaremi et al. introduced the concept of genomic selection, but it took until 2001 for this concept to be more formalized into an approach that could cross from theory into practice (Meuwissen et al., 2001). In less than a decade after this landmark paper, many countries had implemented genomic selection schemes for dairy cattle (Hayes, et al., 2009). The technological breakthrough was low-cost genotyping. However, in the period before genotyping becomes routine practice for dairy farmers, a method to combine information from pedigree relationships has been needed. This was achieved through the development of the H matrix (Misztal et al. 2009), which combined pedigree and genomic information from genotypes. Genomic selection has dramatically changed animal breeding and one area that is benefiting more than others is hard to measure traits, such as methane (Pryce et al., 2020).

### **1.7.2 Genomics and the rate of genetic gain**

A fundamental concept of animal breeding is defining the rate of genetic gain ( $\Delta G$ ) as follows:

$$\Delta G = \frac{r \cdot i \cdot \sigma_a}{L}$$

where  $r$  is the accuracy,  $i$  is the selection intensity,  $\sigma_a$  is the additive genetic variance, and  $L$  is the generation interval. The development and implementation of genomic selection has dramatically increased the rate of genetic improvement by increasing the accuracy of low heritability traits, increasing selection intensity through young sire and heifer selection, and drastically decreasing the generation interval (Shaeffer et al., 2006; Gonzalez-Recio et al., 2014; Chesnais et al., 2016). This impact is especially true for traditional traits that have a large reference population of animals with both phenotypes and genotypes. In these cases, the advantage of genomic selection is that the rate of genetic gain is accelerated by 40-50% (Spelman et al. 2013). For example, Garcia-Ruiz et al (2016) reported that the rate of genetic gain in the American dairy cattle population increased from approximately 50% to 100% for yield traits and from threefold to fourfold in low heritable traits. However, the same level of response has not been observed in novel traits that lack some of the traditional selection criteria.

### 1.7.2 Basic selection index theory

Traditionally, for a trait to be considered in a breeding program it must meet certain selection criteria: 1) have sufficient genetic variation and heritability, 2) have a clear trait definition that can be easily recorded on large number of animals at low cost, and 3) provide economic benefit (Hazel and Lush, 1943). Breeding objectives ( $H$ ) typically focuses on maximizing profit and can be defined as the aggregate of the breeding values for each trait ( $g_n$ ) multiplied by the relative economic value ( $v_n$ ) (Hazel et al., 1994):

$$H = g_1v_1 + g_2v_2 + \dots + g_nv_n$$

In this case, it is assumed that the breeding objective is defined in economic terms and that the economic value is derived from regressing profit on the breeding value of each trait.

Selection indexes are used to maximize genetic progress and efficiently reach the desired goal of the breeding program. This is achieved by combining multiple sources of information each with a specific weight derived based on the relative economic importance of the different traits within the breeding objective. The relative economic value for each trait describes the expected gain in profit due to a one unit of improvement in that trait (Hazel and Lush, 1942). Each trait included in the selection index explains a component of the breeding objective. However, a phenotypic measurement cannot always be collected on all traits in which improvement is desired. Therefore, the traits which meet the selection criteria described above are referred to as index traits or selection criteria traits. For example, somatic cell count was historically used as the index trait to improve mastitis resistance due to the challenge of obtaining detailed mastitis records. The weights of these index traits ( $b$ ) are derived and are defined as:

$$b = P^{-1}Ga$$

where  $P$  is the covariance matrix among the breeding values in the index,  $G$  is the covariance matrix between breeding values and the aggregate true genotype, and  $a$  is a vector of economic value of each trait. When the breeding values are estimated using multiple-trait Best Linear Unbiased Prediction evaluation, the derived weights are equivalent to the relative economic value as  $P = G$ . These weights may then be applied to the respective traits to form an index ( $I$ ) (Hazel 1943):

$$I_i = b_1x_1 + b_2x_2 + \dots + b_nx_n$$

where  $x_n$  is the estimated breeding value and  $b_n$  is the derived weights as described above. This aggregate value represents the animal's cumulative breeding value and can be used to objectively compare animals for many traits simultaneously.

The application of such a well-constructed selection index is more efficient than single-trait selection or simultaneous selection for several traits based on predetermined independent culling levels (Hazel and Lush, 1942). Smith (1983) concluded that the efficiency of a selection index can be maintained, even with changes in economic

weights, unless: 1) important traits are omitted from, or unimportant traits are included in the index or 2) the direction of selection is reversed for an important trait.

### **1.7.3 Developing selection criteria for traits with non-economic value**

As breeding programs are widening to include other objectives beyond profit, these criteria must also be updated. Environmental traits do not necessarily need to be assessed on solely their potential economic impact, but also their environmental impact or societal value, such as the non-economic cost to maintain the industry's social license to operate. Methods have been explored and implemented which attempt to place emphasis on traits based on non-economic stances (Nielsen and Amer, 2007).

Although carbon markets do exist globally, the type of market (carbon tax, carbon credit, etc.) implemented is generally country and region specific, and rarely do open markets that allow for inclusive trading exist. Additionally, the carbon price which may be used to dictate an economic value for methane emissions is also inconsistent. It is unclear when these attributes of current carbon markets may change, therefore, it is important that the non-economic benefits of reducing methane emissions are accounted for in future candidate trait selection criteria. A framework exists to devise nonmarket values for traits under selection (Nielsen et al. 2005), taking into consideration consumer willingness to pay for attributes under genetic control that have perceived environmental or animal welfare value. It is also possible to devise indices that have either a desired outcome, or selection response, or restrict the change in a trait. Other aspects affecting breeding objectives will become more important, for example, as the growing human population places more pressure on limited resources and global changes lead to more extreme conditions in which to manage livestock. There is also a need to recognise increased consumer awareness of animal welfare and farming conditions. Future breeding goals should adapt to these considerations by including economic, societal and environmental considerations simultaneously (Boichard and Brochard 2012; Martin-Collado et al. 2015).

## 1.8 Trait definition

Due to the multitude of phenotypic measurement techniques and difficulty in identifying the best trait for selection, uncertainty exists in determining the direct and indirect effects of selecting for animals in terms of sustainability and environmental impact (Hagemann et al., 2011). Developing a breeding program based on such uncertainty is challenging, as the full impact of selecting for a methane trait is unknown and different methane trait definitions may have contrasting impacts. For the Australian dairy industry, the goal is to improve the overall sustainability of dairy production by reducing methane emissions without negatively affecting economically important traits. This may be achieved by identifying and including a methane trait in the national index that will most effectively reach this overall breeding objective.

There are multiple definitions of methane commonly used to measure and monitor methane emissions, as summarized by de Haas et al. (2017; Table 1). Gross methane, or methane production, is defined as the liters or grams of methane per day. This definition is widely used by emissions recording systems and is currently adapted for national emission inventories and government GHG reduction programs (IPPC, 2016). While this definition is favourable for measuring and recording purposes, it is unfavourably correlated to feed intake and production traits, making it less desired for livestock breeding programs (de Haas et al., 2017). Methane yield describes the unit of methane produced per unit of dry matter intake and attempts to describe the emissions conversion efficiency of the animal and takes advantage of the strong relationship which exist between methane emissions and feed intake (Herd et al., 2013). Methane intensity is the unit of methane produced per unit of product, such as milk and its components. This definition is commonly used in the agriculture industry as it considers that the industry must increase production to feed a growing population (de Haas et al., 2017). Methane yield and methane intensity are both ratio traits, which are challenging to include in selection indexes and breeding programs. When predicting the response to selection, it is difficult to determine which component trait (denominator or

numerator) is the driving force, especially for traits with similar heritability (Gunsett, 1984). To overcome this challenge, residual traits were introduced as a linearization of ratio traits (Kennedy et al., 1993). Residual methane is defined as the difference between an animal's expected and actual methane production and is typically estimated by correcting methane production for traits of interest.

Residual methane may be considered a linearization of methane yield when corrected for feed intake and a linearization of methane intensity when corrected for production level. The linear characteristics of residual methane are more favourable for inclusion in breeding programs compare to its ratio counterparts (VanRaden et al., 2018). However, a residual trait definition can be conceptually challenging to explain to producers as a more negative number is favourable.

**Table 1:** Methane emission trait definitions

Trait	Definition	Strength	Weakness
Methane production	L/d or g/d	Linear trait	Correlated with DMI and ECM
Methane intensity	per kg of output (ECM, protein, fat)	Supports increased production	Ratio trait
Methane yield	per kg of DMI	Supports efficiency in energy partitioning	Ratio trait
Residual methane production	L/d or g/d	Linear trait corrected for traits that influence methane production	Challenging to explain to farmers

\*adapted from de Haas et al. (2017)

Studies have shown that methane is a suitable candidate for genetic selection with coefficients of genetic variation reported between 0.10 and 0.15, and heritability estimates ranging from 0.10 to 0.42 in dairy cattle, depending on the methane trait under investigation (Table 2). Moderate to high genetic correlations have been reported between the various methane traits definitions (Breider et al 2019), as well as between methane traits defined at different stages of lactation (de Haas et al., 2011). This suggests that selection for one of the methane traits may results in genetic progress in

all methane trait definitions. However, further investigation into the different definitions of methane and their impact on other important traits is still needed.

**Table 2:** Heritability estimates for methane emission traits

Source	Country	Method	Trait	No. animals	$h^2$ (SE)
Pszczola et al 2017	Poland	FTIR <sup>1</sup>	Methane production	365	0.23 (0.08) -0.30 (0.12)
Breider et al., 2019	UK	Guardian <sup>2</sup>	Methane production	184	0.12 (0.16) - 0.45 (0.11)
Lassen and Løvendahl, 2016	Denmark	FTIR	Methane production	1745	0.21 (0.06)
			Methane:CO2 ratio	3121	0.16 (0.04)
			Methane intensity	1745	0.21 (0.06)
Manzanilla-Pech et al., 2021	Multi-national	Multiple methods	Methane production	2990	0.21 (0.04)
			Methane yield	2990	0.30 (0.04)
			Methane intensity	2990	0.38 (0.04)
			Residual methane	2990	0.16 (0.04)
de Haas et al., 2011	Netherlands	Energy Predi. Eqn <sup>3</sup>	Methane production	1698	0.35 (0.10) - 0.58 (0.12)
Bittante and Cecchinato, 2019	Italy	Fatty Acid Profiles	Predicted methane production	1091	0.20 (NA)
			Predicted methane yield	1091	0.10 (NA)
			Predicted methane intensity	1091	0.01 (NA)
López-Paredes et al., 2020	Spain	Guardian	Methane production	1501	0.12 (0.04)
			Methane concentration	1501	0.11 (0.03)

<sup>1</sup> Analyzed on breath samples using the Fourier transformed infrared spectroscopy (FTIR) analyzer (GASMET 4030, Gasmeter Technologies Oy, Helsinki, Finland)

<sup>3</sup> Analyzed on breath samples using an infrared CH<sub>4</sub> analyzer (Guardian Plus; Edinburgh Instruments Ltd., Livingston, UK).

<sup>2</sup> Methane was estimated using an energy prediction equation. Methane (g/d) = feed intake (kg of DM/d) × 18.4 (MJ/kg of DM)/0.05565 (MJ/g) × 0.06 × 1 + [2.38 – level of intake (multiples of maintenance level)] × 0.04

### 1.8.2 Genetic correlation with other traits

Few studies have recorded genetic correlations between the various methane trait definitions and other relevant traits of interest in dairy cattle. From a biological perspective, the generation of methane is unfavourably associated to production, feed intake, and bodyweight (**BW**). Using MIR predicted phenotypes, Zetouni et al. (2018) reported a significant negative correlation between methane production and body condition score (**BCS**), body depth, chest width and other diseases, and positive correlations with dairy character, calving to first insemination interval, and first to last insemination interval, using the Fourier transformed infrared spectroscopy technique (**FTIR**) on breath samples. Pszczola et al. (2018) also used FTIR on breath samples and reported similar genetic correlations between methane production and fat yield (0.21), milk yield (0.15), chest width (0.15), size (0.15), dairy strength (0.11), and somatic cell count (0.11). Breider et al (2019) reported a negative correlation between liveweight and methane emissions (-0.18); however, no significant genetic correlation existed between methane production and BW due to large standard errors. Similarly, methane production and methane concentration were low to moderately correlated to production traits (0.17-0.31; Lopez-Paredes et al., 2020). Kandel et al (2017) reported a low to moderate genetic correlations between methane production and milk yield (-0.19), fat yield (0.11), and protein yield (-0.21) and moderate to high correlations between methane intensity and milk yield (-0.68), fat yield (-0.21), and protein yield (-0.66). However, these correlations were estimated using MIR predicted phenotypes obtained from milk samples. When Lopez-Paredes et al (2020) estimated the genetic correlation between methane and other traits of interest using Calo's adjustment of correlations between EBVs, methane production and concentration were positively correlated to stature (0.30 and 0.43, respectively), udder depth (0.10 and 0.18), and angularity (0.08 and 0.19). Evidence has also shown a positive, unfavourable genetic correlation between methane traits and fertility (0.17), with animals that emit less methane having longer days open (Lopez-Paredes et al., 2020).

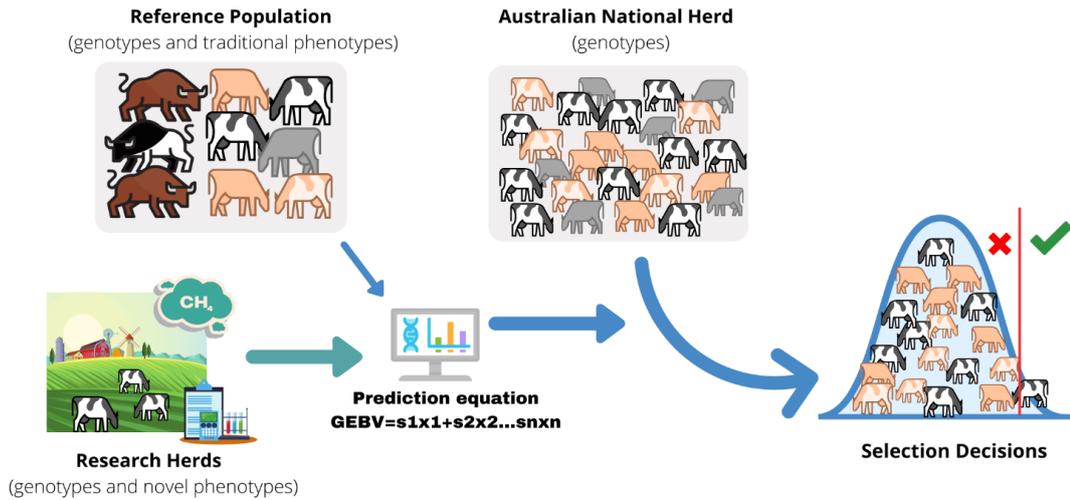
The largest study to date by Manzanilla-Pech et al (2021) reported genetic correlations between multiple definitions of methane, production, maintenance, and efficiency traits using a multi-national, collaborative database built through the Efficient Dairy Genome Project. Milk yield had a moderately positive genetic correlation with methane production (0.29), and a low to moderate negative correlation to methane intensity (-0.57) and residual methane production (-0.05 to -0.22). BW, change in bodyweight ( $\Delta$ BW), and metabolic bodyweight (MBW) were not significantly correlated to methane yield, methane intensity and residual methane. However, methane production had a positive genetic correlation with BW (0.65),  $\Delta$ BW (0.32), and MBW (0.65). BSC had a low correlation to methane production (0.11), methane intensity (-0.04), and residual methane (-0.14), but had a moderate, positive correlation to methane yield (0.46). Dry matter intake had an unfavourable genetic correlation to methane production (0.42), and a minimal to favourable genetic correlation with methane intensity (-0.18), residual methane (-0.02), methane yield (-0.35). Manzanilla-Pech et al. (2021) also noted high correlations between methane production across days in milk, and moderate to high genetic correlations between lactations (0.48-0.91). However, to estimate more accurate genetic correlations between methane traits and other traits of interest in Australia, a national reference population size reasonable for genomic evaluation is required.

### **1.9 Building a primarily female reference population**

Apart from the expense of phenotypic measurements and the challenge of defining economic importance, an additional challenge of including methane in national breeding programs is the composition of the reference population. Chesnais et al., (2016) concluded that while bull reference populations are well suited when phenotypes may easily be collected on a large number of animals, female reference populations are more preferable for expensive or challenging to measure traits. Methane phenotypes are generally recorded on milking cows from research herds within a

limited timeframe, resulting in lower reliabilities estimated from relatively small female only reference population (Gonzalez-Recio 2014). Although many proxy traits have been developed and collaboration is underway through international datasets, methods should be developed to build and maintain a sufficient Australian based reference population.

One approach used by the Australian dairy industry to expand the reference population of novel traits is the introduction of Ginfo herds (DataGene Ltd). These herds are commercial dairy operations that have been identified as maintaining high-quality phenotypic data records. In this scheme, dairy farmers are incentivized through lower genotyping costs to promote the continued recording of more challenging or expensive traits. This large-scale genotyping project has resulted in obtaining the large database of phenotypes and genotypes required to increase the reliabilities of Australian Breeding Values (ABV) and the national indexes. Using this system, traits such as Feed Saved (Pryce et al., 2015), and Heat Tolerance (Nguyen et al., 2018), and metabolic disease resistance (Luke et al., 2019) have been successfully developed. For example, Luke et al. (2019) developed an MIR urea prediction equation that was then successfully applied to increase the accuracy of genomic prediction of urea (van den Berg et al., 2021), using blood urea nitrogen samples from research animals, as well as phenotypes collected from commercial animals on Ginfo farms. This method could also be applied to increase the size of the methane reference population. By combining records from a large network of research farms with novel measurements and proxy traits on commercial animals, a reference population may be built that results in EBVs with accuracies sufficient for implementation (Figure 4).



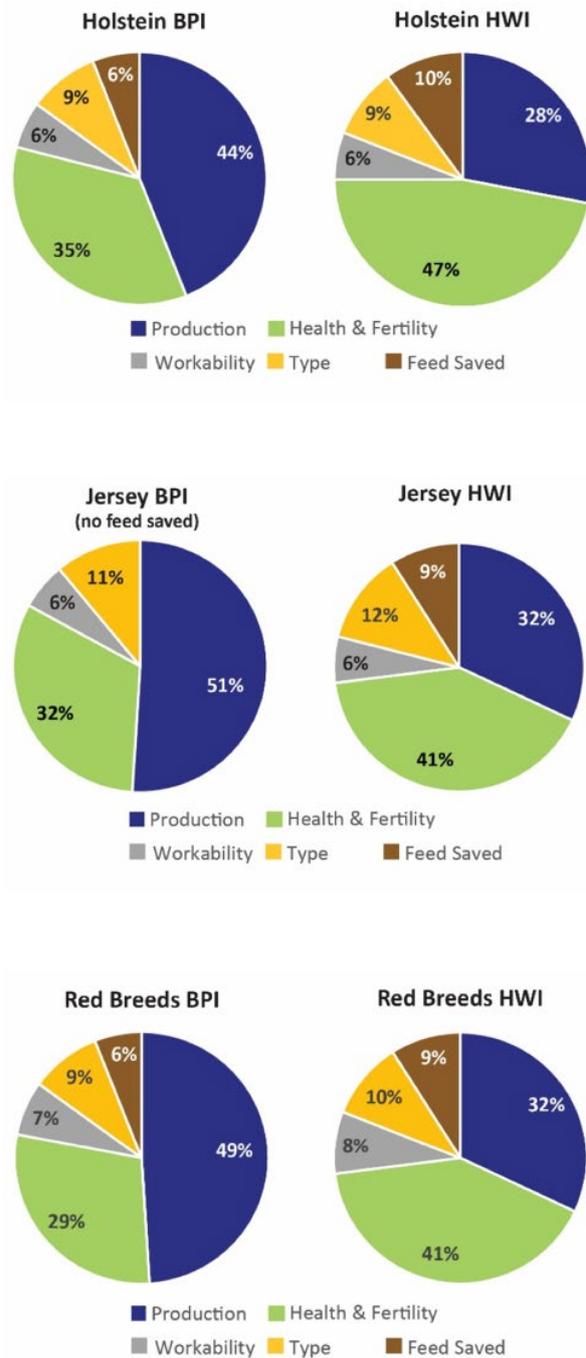
**Figure 4:** Traditional genomic selection uses genotypic and phenotypic information from a reference population to develop prediction equations which can then be applied to the national herd. However, for novel traits such as methane emissions, reference populations of sufficient size are challenging to develop with commercial animals, therefore we rely on research herds and proxy trait development to build reference populations of sufficient size.

### 1.10 Current nation breeding objective

The current Australian national breeding objective includes two national selection indexes (Figure 5): the Balanced Performance Index (BPI) and the Health Weighted Index (HWI). Both consider traits that contribute to cow profitability, farmer preferences and desired gains (Byrne et al., 2016), including biological traits associated with milk production, longevity, fertility, feed efficiency and health. The BPI is an economic index aimed to improve overall profitability on-farm, whereas the HWI includes greater consideration of farmer opinion and subsequently places additional emphasis on fertility, health and efficiency and is aimed at a subset of producers with interests in improving these traits. Details of the index development are given in Byrne et al. (2016), which was recently updated with current industry parameters and economic inputs (Axford et al., 2021). The BPI and HWI are breed specific; therefore, traits included within the indexes received different weights depending on the breed they are applied to (Axford et al., 2021). This allows the indexes to reflect the economic and population averages of each breed.

The HWI reflects the desire of farmers to have genetic selection tools that allow them to select for traits in a multi-trait manner that considers a perceived value on traits that is separate from economics and profit. This willingness to sacrifice potential economic gains for progress in traits for management or social purposes is promising for deriving weights for environmental trait whose benefits are not solely economic based. While there is currently no pre-determined economic value for carbon within Australia, higher social pressure and responsibility to reduce emissions places a new pressure on the industry.

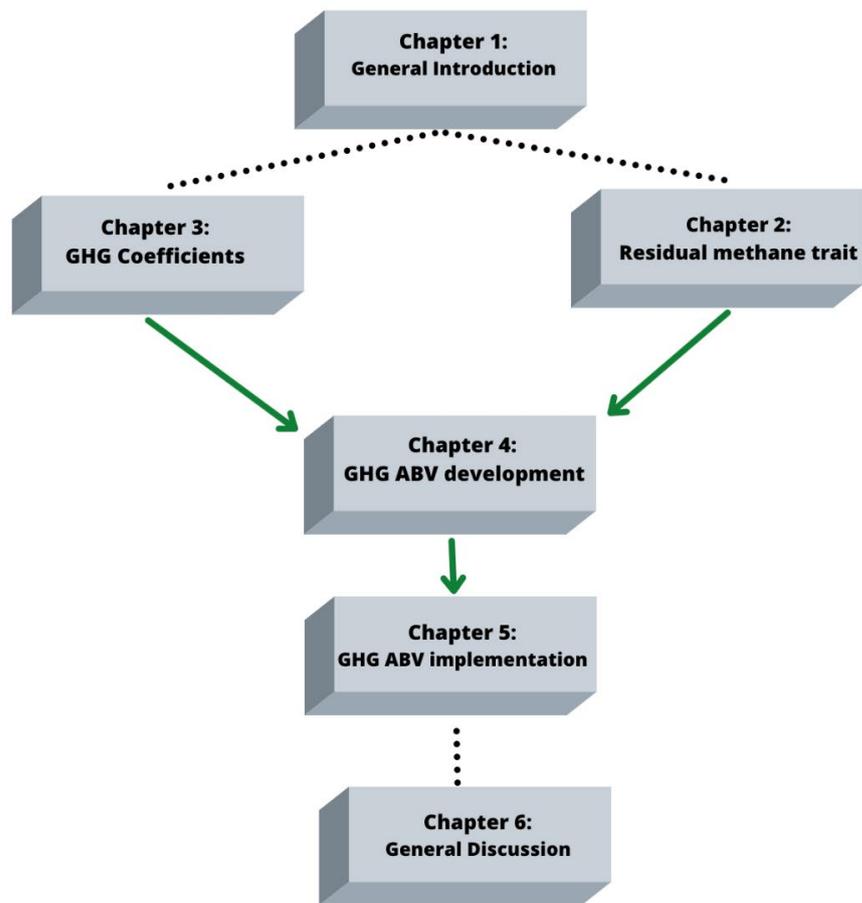
The current Australian dairy industry is committed to sustainability through the Australian Dairy Sustainability Framework (2020); however, there is currently no focus on reducing emissions through the genetics of the national herd. As many of the traits previously identified as having a large environmental impact also have high economic importance, there is an opportunity to introduce a national index that is designed to maximize economic return while lowering environmental impact.



**Figure 5:** Relative emphasis of the Australian dairy national selection indexes for the three major breeds

## 1.11 This thesis

The aim of this thesis is to develop a genetic tool to mitigate GHG emissions that could be easily implemented and understood by dairy farmers. This aim was achieved through four peer-reviewed research chapters published in scientific journals and spanning the topics of trait definition and development to industry level implementation strategies (Figure 6). **Chapter 2** investigated the potential methane candidate trait definitions for the Australian dairy industry, focusing on various definitions of residual methane production. Although this chapter identified a methane production trait corrected for milk production and dry matter intake (i.e. a residual trait) that would be a consideration for the Australian dairy industry, the small dataset available resulted in low accuracies. Therefore, additional methods to quantify the change in emissions due to genetic selection were explored. **Chapter 3** included the calculation of coefficients that describe the impact that current traits under selection have on methane production and methane intensity. These coefficients were used as weights applied to current trait in combination with the low accuracy residual methane trait and were used in **Chapter 4**, which defined options for GHG sub-indexes to reduce emissions. These suggested sub-indexes were then expanded in **Chapter 5** to include a simulated residual methane trait, where I also explored the economic and environmental impact of including the GHG sub-indexes into the BPI. **Chapter 5** also describes how the implementation of the various genetic mitigation strategies might have an impact when considered at the national herd level and draws together the preceding chapters. Finally, **Chapter 6** is a general discussion of the research and considerations on top of those described in **Chapter 5** that may be used for the successful implementation of a genetic tool to reduce GHG emissions and improve the long-term sustainability of the Australian dairy industry.



**Figure 6:** Outline of the general thesis structure, including chapters published in peer-reviewed journals (Chapters 2 to 5) and the general discussion (Chapter 6).

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# **Chapter 2:**

## **Genetic Parameters for Methane Emission Traits in Australian Dairy Cows**

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## Genetic parameters for methane emission traits in Australian dairy cows

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### ABSTRACT

Methane is a greenhouse gas of high interest to the dairy industry, with 57% of Australia's dairy emissions attributed to enteric methane. Enteric methane emissions also constitute a loss of approximately 6.5% of ingested energy. Genetic selection offers a unique mitigation strategy to decrease the methane emissions of dairy cattle, while simultaneously improving their energy efficiency. Breeding objectives should focus on improving the overall sustainability of dairy cattle by reducing methane emissions without negatively affecting important economic traits. Common definitions for methane production, methane yield, and methane intensity are widely accepted, but there is not yet consensus for the most appropriate method to calculate residual methane production, as the different methods have not been compared. In this study, we examined 9 definitions of residual methane production. Records of individual cow methane, dry matter intake (DMI), and energy corrected milk (ECM) were obtained from 379 animals and measured over a 5-d period from 12 batches across 5 yr using the SF<sub>6</sub> tracer method and an electronic feed recording system, respectively. The 9 methods of calculating residual methane involved genetic and phenotypic regression of methane production on a combination of DMI and ECM corrected for days in milk, parity, and experimental batch using phenotypes or direct genomic values. As direct genomic values (DGV) for DMI are not routinely evaluated in Australia at this time, DGV for FeedSaved, which is derived from DGV for residual feed intake and estimated breeding value for bodyweight, were used. Heritability estimates were calculated using univariate models, and correla-

tions were estimated using bivariate models corrected for the fixed effects of year-batch, days in milk, and lactation number, and fitted using a genomic relationship matrix. Residual methane production candidate traits had low to moderate heritability ( $0.10 \pm 0.09$  to  $0.21 \pm 0.10$ ), with residual methane production corrected for ECM being the highest. All definitions of residual methane were highly correlated phenotypically ( $>0.87$ ) and genetically ( $>0.79$ ) with one another and moderately to highly with other methane candidate traits ( $>0.59$ ), with high standard errors. The results suggest that direct selection for a residual methane production trait would result in indirect, favorable improvement in all other methane traits. The high standard errors highlight the importance of expanding data sets by measuring more animals for their methane emissions and DMI, or through exploration of proxy traits and combining data via international collaboration.

**Key words:** methane emission, selection criterion, environmental impact, residual methane, sustainable agriculture

### INTRODUCTION

The world has warmed by about 0.85°C (IPCC, 2013) as greenhouse gases accumulate. When compared with the period between 1986 and 2005, BOM (2015) have modeled that annual average temperatures are projected to increase by 0.6 to 1.3°C by 2030. This may lead to longer times of drought, which is especially damaging to countries such as Australia (BOM, 2015).

Methane has approximately 28 times the global warming potential compared with CO<sub>2</sub>. Dairy cattle account for 20% of the global livestock sector's greenhouse gas emissions, and over half of this is from enteric methane emissions (Gerber et al., 2013). In Australia, the dairy industry contributes only 1.8% to the national emissions inventory through on-farm activities (NGGI,

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2018). The majority of the dairy industry's contribution (57%) is due to the production of enteric methane (UNFCCC, 2018). As a byproduct of feed fermentation in the rumen, enteric methane accounts for 89% of the methane generated by the cow (Broucek, 2014). The long-term sustainability of the dairy industry is dependent on optimizing economic returns and ensuring a social license to operate by minimizing inefficiency and simultaneously increasing environmental consciousness (Cardoso et al., 2016), both of which are results of selecting for reduced methane in dairy cows.

There is increasing pressure being placed on the dairy industry to improve efficiency and increase the sustainability of raising and maintaining cattle for dairy production, despite dairy cattle contributing only a moderate fraction of greenhouse gases emitted by the agriculture sector (Rojas-Downing et al., 2017). During the process of transforming human inedible plant material into energy, 6 to 11% of the energy in feed is lost as methane emissions (Appuhamy et al., 2016). If methane production can be reduced, the saved feed-energy captured by the animal could potentially be redirected to economically important traits such as milk production, BW gain, or both (Yan et al., 2010; Haque, 2018).

Genetic selection is a permanent, cumulative solution to improve the dairy industry's sustainability with minimal additional cost or labor to farmers, especially compared with alternative methods such as feed additives and vaccinations. Although the magnitude of emissions expected to be reduced by selecting for low-methane cows appears small, the dairy industry has a social responsibility to reduce its emissions. Methane is a suitable candidate for reduction through genetic selection, with coefficients of genetic variation reported between 0.10 and 0.15, and heritability estimates ranging from 0.10 to 0.42 in dairy cattle, depending on the methane trait under investigation (de Haas et al., 2011; Pickering et al., 2015; Lassen and Løvendahl, 2016).

Modern breeding objectives in dairy cattle are focused on improving profitability, animal welfare, and environmental impact (Miglior et al., 2017). Although progress has been made in selecting for farmer profit and animal welfare, breeding goals for environmental traits are in their infancy. Indirectly, genetic selection has reduced the environmental impact of dairy cattle through the reduction in rearing periods, resulting from improvements in fertility and survival, and the dilution effect of increased production (Knapp et al., 2014). For the dairy industry, the goal is to improve the overall sustainability of dairy production by reducing methane emissions without negatively affecting economically important traits. This may be achieved by including a methane trait in the national index that is independent

of traits currently included in the overall breeding objective, such as residual methane production (**RMP**).

Residual methane production has been proposed as a possible candidate trait for selection to reduce greenhouse gas emissions from dairy cattle (Manzanilla-Pech et al., 2016; de Haas et al., 2017). Similar to residual feed intake (**RFI**), RMP is estimated as the difference between the actual and predicted methane output based on a subset of measured phenotypes. In contrast to methane production (**MeP**; kg/d), RMP is corrected for traits that highly influence methane outputs. The RMP has statistically favorable properties because it typically uncorrelated with the measured phenotypes used in its calculation and its response to selection may be accurately predicted (de Haas et al., 2017). Ratio traits such as methane yield (**MeY**) (kg/kg of DMI) and methane intensity (**MeI**; kg/kg of output) are undesirable for use in genetic evaluations due to the unexpected responses to selection, as it is unclear which constituent trait drives genetic progress (de Haas et al., 2017).

In beef cattle, RMP is a moderately heritable trait (0.19) and is calculated based on a multiple linear regression of methane production on ADG and live weight (Manzanilla-Pech et al., 2016; Velazco et al., 2016), which are both traits that highly influence methane production through a relationship to feed intake. Identifying traits to correct for in the definition of RMP in dairy cattle is a challenge, with milk production, BW, and feed intake seeming to have the greatest physiological effect on MeP (de Haas et al., 2017). The RMP traits are generally phenotypically independent of the influential traits they are corrected for; however, unaccounted for genetic interactions may still exist. By correcting for direct genomic values (**DGV**) of the influential traits, the calculated RMP traits are genetically independent of the influential traits, an attribute favorable for inclusion of the RMP trait in a selection index (VanRaden et al., 2018). To date, the optimal method to calculate RMP has not yet been established.

The objective of this research was to (1) define candidate RMP traits using a combination of genetic and phenotypic corrections for DMI and ECM, (2) estimate and compare the genetic parameters for the RMP candidate traits, and (3) determine the relationships between these RMP and other methane traits (MeP, MeY, and MeI), as well as ECM and DMI.

## MATERIALS AND METHODS

All experiments in the present study were approved and undertaken in accordance with the Australian Code of Practice for the Care and Use of Animals for

Scientific Purposes (NHMRC, 2013). Approval to proceed was granted by the Agricultural Research and Extension Animal Ethics Committee of the Department of Jobs, Precincts and Regions (DJPR, 475 Mickleham Road, Attwood, Victoria 3049, Australia).

## Phenotypes

**Methane and DMI.** A total of 1,712 individual cow methane and DMI measurements were obtained from 379 cows measured over a 5-d period from 12 experimental batches across 5 yr from 2013 to 2017. Each batch consisted of 22 to 36 animals, and no animals were repeated between batches. Cows were located at the Ellinbank SmartFarm (Ellinbank, Victoria, Australia) and milked twice per day. Measurements for MeP were performed using the SF<sub>6</sub> tracer method previously described by Deighton et al. (2014). Individual feed intakes were measured for the 5-d methane measurement period using an electronic feed recording system (Galagher Animal Management Systems, Hamilton, New Zealand). Records for methane and DMI were averaged to obtain 1 weekly observation per animal for each phenotype to account for day-to-day variation (Moate et al., 2016). Animals were between 68 and 187 DIM at the start of measurement, and across parities 1 to 9. Methane yield was calculated as the 5-d average kg of methane per kg of DMI, and methane intensity as the 5-d average kg of methane per kg of ECM. Daily milk records were available throughout the recording period, with fat and protein percentages obtained through mid-infrared spectroscopy. Energy corrected milk (kg/d) was calculated using Equation 1 (Tyrrell and Reid, 1965) as follows:

$$\text{ECM} = \frac{\text{milk yield (kg)} \times [376 \times \text{fat (\%)} + 209 \times \text{protein (\%)} + 948]}{3,138} \quad [1]$$

In addition to calculating ECM with phenotypes collected as part of the study, we also calculated ECM from DGV so that we could investigate the phenotypic and genetic correction of ECM. Therefore, Equation 1 was adjusted for the use of DGV as follows:

$$\text{ECM} = \frac{\left\{ \left[ 6,861 + \text{DGV}_{\text{milk yield}} \right] \times \left[ 376 \times (4.0 + \text{DGV}_{\text{fat\%}}) + 209 \times (3.3 \times \text{DGV}_{\text{protein\%}}) + 948 \right] \right\}}{3,138}, \quad [2]$$

The DGV for the 379 animals used in this study were estimated as part of the routine genetic evaluation service of DataGene Ltd. (Bundoora, Victoria, Australia) using a data repository that contained 200 million herd test records at the time of this study, and that continues to increase by 2.9 million herd test records annually (Newton et al., 2020). As DataGene does not calculate DGV for ECM as part of the national service, DGV for ECM were calculated using Equation 2, where phenotypic measurements for milk, fat, and protein in Equation 1 were replaced for milk, fat, and protein DGV that were readjusted for the baseline trait means. FeedSaved (**FS**) is derived from DGV for RFI, and from EBV for BW (Pryce et al., 2015).

Descriptive statistics for phenotypes are presented in Table 1. The distributions of residuals were checked for normality, skewness, and kurtosis, and no outliers were detected. Outliers were defined as observations more than 3 standard deviations away from the mean.

## Alternate Definitions of RMP

The aim of assessing these alternate definitions of RMP was to develop an RMP trait that may be easily incorporated in a breeding program and included in a national selection index. To achieve this, 9 selection criteria were considered (Table 2). It is often assumed that there is no correlation between a residual trait

**Table 1.** Descriptive statistics of the data set used in this study

Trait <sup>1</sup>	Unit	No. of cows	Mean	SD	Minimum	Maximum
MeP	g of methane/d	379	469.00	81.03	230.80	753.90
MeY	g of methane/kg of DMI	379	19.00	3.44	10.66	31.00
MeI	g of methane/ kg of ECM	379	18.15	3.82	8.58	32.08
DMI	kg/d	379	24.66	3.18	14.59	32.33
ECM <sup>2</sup>	kg/d	379	25.73	4.08	13.50	39.34
FS	kg	379	39.86	78.62	-191.50	316.40
ECM_BV	kg	379	12.60	69.57	-185.37	187.58

<sup>1</sup>MeP = methane production; MeY = methane yield; MeI = methane intensity; FS = FeedSaved direct genomic value; ECM\_BV = ECM direct genomic value.

<sup>2</sup>Calculated using methods of Tyrrell and Reid (1965).

and the influential traits used in its calculation; however, genetic correlations may still exist. To account for these possible genetic correlations, a correction for the currently available DGV of traits of interest has been proposed (VanRaden et al., 2018). As DGV for DMI are not routinely evaluated in Australia at this time, DGV for FS, the current trait designed to breed for feed efficiency in Australia (Pryce et al., 2015), were used. The FS trait combines the lifetime RFI of the animal, calculated as a weighted average of the calf and lactating cow RFI, and the maintenance energy requirement of the animal estimated through BW (Pryce et al., 2015). Therefore, for individuals with similar milk production EBV, those that save more feed (i.e., that have higher EBV for FS) have lower requirements for feed, predicted using a combination of RFI and BW as a proxy of maintenance.

In total, 9 different definitions of RMP were tested based on a combination of DMI, FS, and ECM corrections. These were:

### 1. Correction of Methane for DMI.

$$\text{RMP}_{\text{DMI}} = \text{MeP} - (b_{\text{DMI}} \times \text{DMI}), \quad [3]$$

where  $\text{RMP}_{\text{DMI}}$  is RMP phenotypically corrected for DMI; MeP is gross methane production (g/d); DMI is measured in kilograms per day; and  $b_{\text{DMI}}$  is the linear regression coefficient of MeP on DMI.

### 2. Correction of Methane for the DGV of FS.

$$\text{RMP}_{\text{FS}} = \text{MeP} - (b_{\text{FS}} \times \text{FS}), \quad [4]$$

where  $\text{RMP}_{\text{FS}}$  is RMP genotypically corrected for FS;  $b_{\text{FS}}$  is the linear regression coefficient of MeP on FS; and MeP is as described above.

### 3. Correction of Methane for DMI and the DGV of FS.

$$\text{RMP}_{\text{DMI,FS}} = \text{MeP} - (b_{\text{DMI}} \times \text{DMI} + b_{\text{FS}} \times \text{FS}), [5]$$

where  $\text{RMP}_{\text{DMI,FS}}$  is RMP phenotypically corrected for DMI and genotypically corrected for FS; and MeP,  $b_{\text{DMI}}$ , DMI,  $b_{\text{FS}}$ , and FS are as described above.

### 4. Correction of Methane for ECM.

$$\text{RMP}_{\text{pECM}} = \text{MeP} - (b_{\text{pECM}} \times \text{ECM}), \quad [6]$$

where  $\text{RMP}_{\text{pECM}}$  is RMP phenotypically corrected for ECM; ECM is measured in kilograms per day;  $b_{\text{pECM}}$  is the linear regression coefficient of MeP on ECM; and MeP is as described above.

### 5. Correction of Methane for the DGV of ECM.

$$\text{RMP}_{\text{gECM}} = \text{MeP} - (b_{\text{gECM}} \times \text{ECM}_{\text{BV}}), \quad [7]$$

**Table 2.** Definitions of 9 residual methane production (RMP) candidate traits

Abbreviation	Equation
$\text{RMP}_{\text{DMI}}^1$	$= \text{MeP} - (b_{\text{DMI}} \times \text{DMI})$
$\text{RMP}_{\text{FS}}^2$	$= \text{MeP} - (b_{\text{FS}} \times \text{FS})$
$\text{RMP}_{\text{DMI,FS}}^3$	$= \text{MeP} - (b_{\text{DMI}} \times \text{DMI} + b_{\text{FS}} \times \text{FS})$
$\text{RMP}_{\text{pECM}}^4$	$= \text{MeP} - (b_{\text{pECM}} \times \text{ECM})$
$\text{RMP}_{\text{gECM}}^5$	$= \text{MeP} - (b_{\text{gECM}} \times \text{ECM}_{\text{BV}})$
$\text{RMP}_{\text{pECM,gECM}}^6$	$= \text{MeP} - (b_{\text{pECM}} \times \text{ECM} + b_{\text{gECM}} \times \text{ECM}_{\text{BV}})$
$\text{RMP}_{\text{DMI,pECM}}^7$	$= \text{MeP} - (b_{\text{DMI}} \times \text{DMI} + b_{\text{pECM}} \times \text{ECM})$
$\text{RMP}_{\text{FS,gECM}}^8$	$= \text{MeP} - (b_{\text{FS}} \times \text{FS} + b_{\text{gECM}} \times \text{ECM}_{\text{BV}})$
$\text{RMP}_{\text{DMI,FS,pECM,gECM}}^9$	$= \text{MeP} - (b_{\text{DMI}} \times \text{DMI} + b_{\text{pECM}} \times \text{ECM} + b_{\text{FS}} \times \text{FS} + b_{\text{gECM}} \times \text{ECM}_{\text{BV}})$

<sup>1</sup> $\text{RMP}_{\text{DMI}}$  = RMP phenotypically corrected for DMI; MeP = gross methane production (g/d); and  $b_{\text{DMI}}$  = the linear regression coefficient of MeP on DMI.

<sup>2</sup> $\text{RMP}_{\text{FS}}$  = RMP genotypically corrected for FS; FS = the FeedSaved direct genomic value;  $b_{\text{FS}}$  = the linear regression coefficient of MeP on FS.

<sup>3</sup> $\text{RMP}_{\text{DMI,FS}}$  = RMP phenotypically corrected for DMI and genotypically corrected for FS.

<sup>4</sup> $\text{RMP}_{\text{pECM}}$  = RMP phenotypically corrected for ECM;  $b_{\text{pECM}}$  = the linear regression coefficient of MeP on ECM.

<sup>5</sup> $\text{RMP}_{\text{gECM}}$  = residual methane production genotypically corrected for ECM;  $\text{ECM}_{\text{BV}}$  = the direct genomic value of ECM;  $b_{\text{gECM}}$  = the linear regression coefficient of MeP on ECM direct genomic value.

<sup>6</sup> $\text{RMP}_{\text{pECM,gECM}}$  = RMP phenotypically and genotypically corrected for ECM.

<sup>7</sup> $\text{RMP}_{\text{DMI,pECM}}$  = RMP phenotypically corrected for DMI and ECM.

<sup>8</sup> $\text{RMP}_{\text{FS,gECM}}$  = residual methane production genotypically corrected for ECM and FS.

<sup>9</sup> $\text{RMP}_{\text{DMI,FS,pECM,gECM}}$  = RMP phenotypically corrected for DMI and ECM and genotypically corrected for ECM and FS.

where  $RMP_{gECM}$  is residual methane production genotypically corrected for ECM;  $ECM_{BV}$  is the DGV of ECM;  $b_{gECM}$  is the linear regression coefficient of MeP on ECM direct genomic value; and MeP is as described above.

### 6. Correction of Methane for ECM and the DGV of ECM.

$$RMP_{pECM,gECM} = \text{MeP} - (b_{pECM} \times \text{ECM} + b_{gECM} \times \text{ECM}_{BV}), \quad [8]$$

where  $RMP_{pECM,gECM}$  is RMP phenotypically and genotypically corrected for ECM; and MeP,  $b_{pECM}$ , ECM,  $b_{gECM}$ , and  $ECM_{BV}$  are as described above.

### 7. Correction of Methane for DMI and ECM.

$$RMP_{DMI,ECM} = \text{MeP} - (b_{DMI} \times \text{DMI} + b_{pECM} \times \text{ECM}), \quad [9]$$

where  $RMP_{DMI,ECM}$  is RMP phenotypically corrected for DMI and ECM; and MeP,  $b_{DMI}$ , DMI,  $b_{pECM}$ , and ECM are as described above.

### 8. Correction of Methane for the DGV of ECM and FS.

$$RMP_{FS,gECM} = \text{MeP} - (b_{FS} \times \text{FS} + b_{gECM} \times \text{ECM}_{BV}), \quad [10]$$

where  $RMP_{FS,gECM}$  is residual methane production genotypically corrected for ECM and FS; and MeP,  $b_{FS}$ , FS,  $b_{gECM}$ , and  $ECM_{BV}$  are as described above.

### 9. Correction of Methane for ECM, DMI, and the DGV of ECM and FS.

$$RMP_{DMI,FS,pECM,gECM} = \text{MeP} - (b_{DMI} \times \text{DMI} + b_{pECM} \times \text{ECM} + b_{FS} \times \text{FS} + b_{gECM} \times \text{ECM}_{BV}), \quad [11]$$

where  $RMP_{DMI,FS,pECM,gECM}$  is RMP phenotypically corrected for DMI and ECM and genotypically corrected for ECM and FS; and MeP,  $b_{DMI}$ , DMI,  $b_{pECM}$ , ECM,  $b_{FS}$ , FS,  $b_{gECM}$ , and  $ECM_{BV}$  are as described above.

## Genomic Analysis

Genotypes for the 379 animals used in this study were from the routine genomic evaluation system of DataGene Ltd., comprising 47,162 single nucleotide polymorphism markers; the editing procedure used is similar to Erbe et al. (2012). A genomic relationship matrix ( $\mathbf{G}$ ) was constructed following the method of Yang et al. (2010). Principal component analysis of  $\mathbf{G}$

was performed in R using the *prcomp* function in the stats package to confirm that no subpopulations were present in the data set (R Core Team, 2013).

## Estimating Genetic Parameters

Univariate and bivariate analysis were performed to estimate genetic parameters using the ASREML 4.1 software program (Gilmour et al., 2015). Genetic parameters were estimated for RMP and associated traits using models that corrected for the fixed effects of lactation number (where lactations numbers were 1, 2, 3, and 4+, hence merging all lactations after the third lactation), DIM as the midpoint of the 5-d test period, and year  $\times$  batch interaction. Linear and quadratic effects were tested for DIM, DMI, and ECM. Only the linear effect was significant ( $P < 0.05$ ) for ECM and DMI, with the linear and quadratic effects significant for DIM.

Heritability and variance components were estimated for each trait using the same univariate linear animal model for all traits as follows:

$$y_{ijkl} = \mu + YB_i + DIM_j + DIM_j^2 + LN_k + g_l + e_{ijkl}$$

where  $y_{ijkl}$  is each trait (MeP, MeY, MeI, RMP definitions 1–9, ECM or DMI);  $\mu$  is the overall mean;  $YB_i$  is the effect of  $i$ th year  $\times$  batch interaction;  $DIM_j$  is the linear effect;  $DIM_j^2$  the quadratic effect of  $j$ th DIM;  $LN_k$  is the effect of  $k$ th lactation;  $g_l$  is the random additive genetic effect of  $l$ th animal,  $g_l \sim N(0, \mathbf{G}\sigma_g^2)$ , where  $\mathbf{G}$  is the genomic relationship matrix and  $\sigma_g^2$  is the additive genetic variance; and  $e_{ijkl}$  is the random residual effect,  $e_{ijkl} \sim N(0, \mathbf{I}\sigma_e^2)$ , where  $\mathbf{I}$  is the identity matrix and  $\sigma_e^2$  is error variance. As all records originated from the same season (spring), we did not include a month or season effect in the model.

Bivariate analyses were performed to estimate genetic and phenotypic correlations between the 9 RMP trait definitions, the 9 RMP traits and other methane candidate traits (MeP, MeY, MeI), and all methane candidate traits and ECM and DMI. The same model as previously described was used to perform the analysis for each pair of traits.

## RESULTS

The aim of this study was to define and compare 9 candidate RMP traits. The defined traits were developed by correcting for traits of influence on MeP and for compatibility with the national selection index. Therefore, the proposed definitions of RMP are a com-

bination of phenotypic and genetic corrections for DMI and ECM. The relationship between the 9 RMP trait definitions and other methane candidate traits, ECM, and DMI were then compared for identification of a future selection criterion.

### Heritability Estimates

Genetic parameters for RMP candidate traits are shown in Table 3. Heritability estimates of RMP traits were low to moderate with  $RMP_{FS,gECM}$  and  $RMP_{gECM}$  being the least heritable ( $0.11 \pm 0.10$ ) and  $RMP_{pECM}$  being the most heritable trait ( $0.21 \pm 0.11$ ). Heritability estimates for  $RMP_{DMI}$ ,  $RMP_{FS,DMI}$ ,  $RMP_{DMI,pECM}$ , and  $RMP_{DMI,FS,pECM,gECM}$  were all  $0.18 \pm 0.11$ , and were  $0.15 \pm 0.11$  and  $0.19 \pm 0.11$  for  $RMP_{FS}$  and  $RMP_{pECM,gECM}$ , respectively. Heritability for MeP, MeY, MeI, DMI and ECM was estimated as  $0.16 \pm 0.11$ ,  $0.23 \pm 0.12$ ,  $0.33 \pm 0.12$ ,  $0.33 \pm 0.13$ , and  $0.29 \pm 0.13$ , respectively.

### Genetic Correlations Between RMP Traits

High phenotypic and genetic correlations were observed between RMP traits (Table 3). The genetic correlations between  $RMP_{DMI}$  and  $RMP_{FS}$ ,  $RMP_{DMI}$  and  $RMP_{DMI,FS}$ , and  $RMP_{FS}$  and  $RMP_{DMI,FS}$  were  $0.77 \pm 0.18$ ,  $0.99 \pm$  not estimable, and  $0.78 \pm 0.18$ , respectively. The genetic correlations between traits corrected for ECM was  $0.99 \pm 0.09$  between  $RMP_{gECM}$  and  $RMP_{pECM}$ ,  $0.99 \pm 0.001$  between  $RMP_{pECM}$  and  $RMP_{pECM,gECM}$ , and  $0.99 \pm 0.07$  between  $RMP_{gECM}$  and  $RMP_{pECM,gECM}$ . High genetic correlations were observed ranging from  $0.98 \pm 0.02$  to  $0.99 \pm 0.002$  between  $RMP_{DMI,pECM}$ ,  $RMP_{FS,gECM}$ , and  $RMP_{DMI,FS,pECM,gECM}$ .

### Genetic Correlations Between Phenotypically and Genotypically Corrected Traits

Genetic correlations between all RMP traits corrected for trait DGW ranged from  $0.87 \pm 0.12$  to  $0.99 \pm 0.001$  (Table 3). Genetic correlations between traits phenotypically corrected for DMI, ECM, or both ranged from  $0.81 \pm 0.12$  to  $0.99 \pm 0.001$ . The  $RMP_{pECM,gECM}$  was highly correlated with other traits corrected both phenotypically and genetically, with a genetic correlation with  $RMP_{DMI,FS,pECM,gECM}$  and  $RMP_{DMI,FS}$  of  $0.83 \pm 0.11$  and  $0.83 \pm 0.12$ , respectively. However, the genetic correlation between  $RMP_{DMI,FS,pECM,gECM}$  and  $RMP_{DMI,FS}$  was stronger ( $0.99 \pm 0.002$ ). Standard errors were not possible to obtain when estimating the genetic correlation between  $RMP_{FS,DMI}$  and  $RMP_{DMI,pECM}$  and the phenotypic correlation between  $RMP_{DMI,FS,pECM,gECM}$  and  $RMP_{DMI}$ , as these 2 correlations were very close to 1.

**Table 3.** Genetic parameters for measures of residual methane production; heritability<sup>1</sup> is presented on the diagonal with genetic correlation above and phenotypic correlations below

Item <sup>2</sup>	$\sigma_g$	$RMP_{DMI}$	$RMP_{FS}$	$RMP_{DMI,FS}$	$RMP_{pECM}$	$RMP_{gECM}$	$RMP_{pECM,gECM}$	$RMP_{DMI,pECM}$	$RMP_{FS,gECM}$	$RMP_{DMI,FS,pECM,gECM}$
$RMP_{DMI}$	28.40	0.18 ± 0.11	0.77 ± 0.18	0.99 ± NE <sup>3</sup>	0.81 ± 0.12	0.87 ± 0.12	0.83 ± 0.12	0.99 ± 0.00	0.98 ± 0.12	0.99 ± 0.00
$RMP_{FS}$	26.48	0.87 ± 0.01	0.15 ± 0.11	0.78 ± 0.18	0.92 ± 0.08	0.87 ± 0.12	0.82 ± 0.12	0.78 ± 0.17	0.90 ± 0.09	0.71 ± 0.21
$RMP_{DMI,FS}$	27.14	0.99 ± NE <sup>3</sup>	0.87 ± 0.01	0.18 ± 0.11	0.81 ± 0.12	0.93 ± 0.13	0.83 ± 0.12	0.99 ± NE	0.98 ± 0.12	0.99 ± 0.00
$RMP_{pECM}$	32.07	0.92 ± 0.01	0.93 ± 0.01	0.92 ± 0.01	0.21 ± 0.11	0.99 ± 0.09	0.99 ± 0.01	0.82 ± 0.12	0.99 ± 0.10	0.80 ± 0.13
$RMP_{gECM}$	24.78	0.98 ± 0.00	0.98 ± 0.00	0.88 ± 0.01	0.94 ± 0.01	0.11 ± 0.10	0.99 ± 0.07	0.95 ± 0.12	0.98 ± 0.02	0.93 ± 0.12
$RMP_{pECM,gECM}$	27.27	0.92 ± 0.01	0.93 ± 0.01	0.93 ± 0.01	0.99 ± 0.00	0.95 ± 0.00	0.19 ± 0.11	0.84 ± 0.11	0.98 ± 0.07	0.83 ± 0.11
$RMP_{DMI,pECM}$	28.22	0.99 ± 0.00	0.87 ± 0.01	0.99 ± 0.00	0.93 ± 0.01	0.88 ± 0.01	0.93 ± 0.01	0.18 ± 0.11	0.99 ± 0.12	0.99 ± 0.00
$RMP_{FS,gECM}$	23.58	0.88 ± 0.01	0.99 ± 0.00	0.88 ± 0.01	0.94 ± 0.00	0.99 ± 0.00	0.95 ± 0.01	0.89 ± 0.01	0.11 ± 0.10	0.98 ± 0.12
$RMP_{DMI,FS,pECM,gECM}$	27.77	0.99 ± NE	0.87 ± 0.01	0.99 ± NE	0.93 ± 0.01	0.88 ± 0.01	0.93 ± 0.01	0.99 ± 0.00	0.88 ± 0.01	0.18 ± 0.11

<sup>1</sup>Heritability estimates were calculated using univariate models and correlations were estimated using bivariate models, where  $\sigma_g$  is the genetic standard deviation.

<sup>2</sup> $RMP_{DMI}$  = residual methane production phenotypically corrected for DMI;  $RMP_{FS}$  = residual methane production genotypically corrected for FeedSaved (FS);  $RMP_{DMI,FS}$  = residual methane genotypically corrected for FS and phenotypically corrected for DMI;  $RMP_{pECM}$  = residual methane production phenotypically corrected for ECM;  $RMP_{gECM}$  = residual methane production genotypically corrected for ECM breeding values;  $RMP_{pECM,gECM}$  = residual methane genotypically and phenotypically corrected for ECM;  $RMP_{DMI,pECM}$  = residual methane phenotypically corrected for DMI and ECM;  $RMP_{FS,gECM}$  = residual methane genotypically corrected for FS and ECM;  $RMP_{DMI,FS,pECM,gECM}$  = residual methane genotypically corrected for FS, phenotypically corrected for DMI, and genotypically and phenotypically corrected for ECM.

<sup>3</sup>NE = not estimable.

**Table 4.** Genetic (rg) and phenotypic (rp) correlations between gross methane, methane yield, methane intensity, and residual methane traits; parameters were estimated using bivariate models<sup>1</sup>

Residual methane trait <sup>2</sup>	rg MeP	rp MeP	rg MeY	rp MeY	rg MeI	rp MeI
RMP <sub>DMI,FS,pECM,gECM</sub>	0.66 (0.23)	0.87 (0.01)	0.95 (0.04)	0.95 (0.01)	0.82 (0.14)	0.79 (0.02)
RMP <sub>FS,gECM</sub>	0.88 (0.09)	0.98 (0.002)	0.79 (0.25)	0.72 (0.03)	0.97 (0.37)	0.66 (0.03)
RMP <sub>DMI,pECM</sub>	0.72 (0.19)	0.87 (0.01)	0.93 (0.05)	0.95 (0.01)	0.80 (0.15)	0.80 (0.02)
RMP <sub>pECM,gECM</sub>	0.81 (0.13)	0.93 (0.01)	0.66 (0.20)	0.81 (0.02)	0.91 (0.09)	0.85 (0.01)
RMP <sub>gECM</sub>	0.90 (0.08)	0.99 (0.00)	0.73 (0.26)	0.72 (0.03)	0.86 (0.23)	0.66 (0.03)
RMP <sub>pECM</sub>	0.89 (0.08)	0.93 (0.01)	0.60 (0.23)	0.81 (0.02)	0.86 (0.09)	0.85 (0.01)
RMP <sub>DMI,FS</sub>	0.71 (0.20)	0.87 (0.01)	0.94 (0.05)	0.95 (0.01)	0.79 (0.15)	0.78 (0.02)
RMP <sub>FS</sub>	0.97 (0.02)	0.99 (0.00)	0.52 (0.30)	0.70 (0.03)	0.64 (0.25)	0.63 (0.03)
RMP <sub>DMI</sub>	0.72 (0.20)	0.87 (0.01)	0.93 (0.04)	0.95 (0.01)	0.79 (0.15)	0.79 (0.02)

<sup>1</sup>MeP = methane production; MeY = methane yield; MeI = methane intensity.

<sup>2</sup>RMP<sub>DMI</sub> = residual methane production phenotypically corrected for DMI; RMP<sub>FS</sub> = residual methane production genotypically corrected for FeedSaved (FS); RMP<sub>DMI,FS</sub> = residual methane genotypically corrected for FS and phenotypically corrected for DMI; RMP<sub>pECM</sub> = residual methane production phenotypically corrected for ECM; RMP<sub>gECM</sub> = residual methane production genotypically corrected for ECM breeding values; RMP<sub>pECM,gECM</sub> = residual methane genotypically and phenotypically corrected for ECM; RMP<sub>DMI,pECM</sub> = residual methane phenotypically corrected for DMI and ECM; RMP<sub>FS,gECM</sub> = residual methane genotypically corrected for FS and ECM; RMP<sub>DMI,FS,pECM,gECM</sub> = residual methane genotypically corrected for FS, phenotypically corrected for DMI, and genotypically and phenotypically corrected for ECM.

### Correlations Between Methane Candidate Traits

The genetic and phenotypic correlation between measures of RMP and MeP, MeY, and MeI were moderate to high ( $\geq 0.52 \pm 0.30$  for genetic and  $\geq 0.63 \pm 0.03$  for phenotypic correlations, respectively; Table 4). The MeP had the strongest genetic correlation with RMP<sub>FS</sub> ( $0.97 \pm 0.02$ ), and the weakest with RMP<sub>DMI,FS,pECM,gECM</sub> ( $0.66 \pm 0.23$ ). The MeY had the strongest genetic correlation with RMP<sub>DMI,FS,pECM,gECM</sub> ( $0.95 \pm 0.04$ ) and weakest with RMP<sub>FS</sub> ( $0.52 \pm 0.30$ ). The MeI had the highest genetic correlation with RMP<sub>FS,gECM</sub> ( $0.97 \pm 0.37$ ) and, considering standard errors, RMP<sub>pECM,gECM</sub> ( $0.91 \pm 0.09$ ) and weakest with RMP<sub>FS</sub> of  $0.64 \pm 0.25$ .

### Correlations of Methane Definitions with ECM and DMI

**Genetic Correlations.** The only methane candidate trait that had a significant genetic correlation with DMI was MeY ( $-0.60 \pm 0.25$ ), and with ECM was MeI ( $-0.73 \pm 0.18$ ; Table 5). Standard errors for genetic correlations between methane candidate traits and ECM and DMI were high; therefore, the genetic correlations will not be considered further.

**Phenotypic Correlations.** The phenotypic correlations between DMI and MeP, MeY, and MeI were  $0.49 \pm 0.04$ ,  $-0.27 \pm 0.05$ , and  $-0.11 \pm 0.05$ , respectively (Table 4). The RMP traits that were phenotypically

**Table 5.** Genetic (rg) and phenotypic (rp) correlations between ECM and DMI with gross methane, methane yield, methane intensity, and residual methane traits; parameters were estimated using bivariate models and standard errors are presented in parentheses

Trait <sup>1</sup>	rg DMI	rp DMI	rg ECM	rp ECM
MeP	0.42 (0.30)	0.49 (0.04)	-0.08 (0.39)	0.35 (0.05)
MeY	-0.60 (0.25)	-0.27 (0.05)	-0.53 (0.33)	-0.17 (0.05)
MeI	-0.29 (0.26)	-0.11 (0.05)	-0.73 (0.18)	-0.47 (0.04)
RMP <sub>DMI</sub>	-0.29 (0.36)	-0.01 (0.05)	-0.37 (0.41)	0.01 (0.05)
RMP <sub>FS</sub>	0.31 (0.35)	0.47 (0.04)	-0.05 (0.46)	0.34 (0.05)
RMP <sub>DMI,FS</sub>	-0.30 (0.36)	-0.01 (0.05)	-0.38 (0.41)	0.01 (0.05)
RMP <sub>pECM</sub>	0.14 (0.34)	0.26 (0.05)	-0.50 (0.38)	-0.02 (0.05)
RMP <sub>gECM</sub>	0.05 (0.46)	0.44 (0.04)	-0.41 (0.62)	0.30 (0.05)
RMP <sub>pECM,gECM</sub>	0.01 (0.36)	0.26 (0.05)	-0.49 (0.38)	-0.00 (0.05)
RMP <sub>DMI,pECM</sub>	-0.29 (0.36)	-0.01 (-0.05)	-0.38 (0.40)	-0.00 (0.05)
RMP <sub>FS,gECM</sub>	-0.03 (0.49)	0.43 (0.04)	-0.53 (0.70)	0.29 (0.04)
RMP <sub>DMI,FS,pECM,gECM</sub>	-0.36 (0.36)	-0.01 (0.05)	-0.46 (0.41)	-0.00 (0.05)

<sup>1</sup>MeP = methane production; MeY = methane yield; MeI = methane intensity; RMP<sub>DMI</sub> = residual methane production phenotypically corrected for DMI; RMP<sub>FS</sub> = residual methane production genotypically corrected for FeedSaved (FS); RMP<sub>DMI,FS</sub> = residual methane genotypically corrected for FS and phenotypically corrected for DMI; RMP<sub>pECM</sub> = residual methane production phenotypically corrected for ECM; RMP<sub>gECM</sub> = residual methane production genotypically corrected for ECM breeding values; RMP<sub>pECM,gECM</sub> = residual methane genotypically and phenotypically corrected for ECM; RMP<sub>DMI,pECM</sub> = residual methane phenotypically corrected for DMI and ECM; RMP<sub>FS,gECM</sub> = residual methane genotypically corrected for FS and ECM; RMP<sub>DMI,FS,pECM,gECM</sub> = residual methane genotypically corrected for FS, phenotypically corrected for DMI, and genotypically and phenotypically corrected for ECM.

corrected for DMI,  $RMP_{DMI}$ ,  $RMP_{DMI,ECM}$ , and  $RMP_{DMI,FS,pECM,gECM}$ , had no significant correlations to DMI. In contrast,  $RMP_{FS}$  had a phenotypic correlation of  $0.47 \pm 0.04$  with DMI. Energy corrected milk was phenotypically correlated with MeP ( $0.35 \pm 0.05$ ), MeY ( $-0.17 \pm 0.05$ ), and MeI ( $-0.47 \pm 0.04$ ). Phenotypic correlations were found between  $RMP_{gECM}$  and ECM ( $0.30 \pm 0.05$ ), but were not significant between ECM and  $RMP_{pECM}$ ,  $RMP_{DMI,pECM}$ ,  $RMP_{DMI,FS,pECM,gECM}$ , and  $RMP_{DMI}$ . All other calculated phenotypic and genotypic correlations between ECM, DMI, and the various RMP traits were not significant ( $P > 0.05$ ).

## DISCUSSION

In this research, we compared 9 methods of calculating RMP traits, adjusting phenotypically and genotypically for influential traits (DMI and ECM). The estimated genetic parameters for the defined RMP traits were then compared for all RMP traits as well as the other methane candidate traits. The overall aim was to identify a candidate methane trait for inclusion in the Australian national breeding program that would reduce the environmental impact of dairy cattle without severely affecting other valuable traits such as production, health, and fertility. The trait should also have the ability to be measured on a large number of individuals and be computationally simple.

### Including Methane in Breeding Programs

As decreasing net emissions may result in a decrease in overall production or general efficiency (Hayes et al., 2013), there is a lack of consensus on management strategies to lower the environmental impact of dairy cattle. However, net emissions will continue to grow proportionally to total dairy production, which is linked to increasing consumer demand for dairy products as the human population increases. Therefore, reducing MeP by reducing milk production is undesirable.

Traditionally, there are many accepted trait definitions to consider for methane when exploring candidate traits for genetic selection, including gross, ratio, and residual. If there is an implied or real economic cost attached to MeP, it might seem simplest to include MeP in the breeding objective and to publish an EBV for this trait. By considering the economic weights of other traits and the genetic parameters of all traits, an efficient selection index could be derived. However, advising dairy producers to select for a trait (i.e., low MeP) that is unfavorably correlated with milk yield might lead to confusion and lack of adoption. Therefore, we have investigated composite traits that could be included in the index without creating this problem.

Ratio traits allow for the consideration of multiple components in a trait definition that is convenient for industry interpretation. However, the application of ratio traits in genetics is contested (Berry and Crowley, 2013) because the complex statistical properties of ratio traits may lead to unfavorable correlations (i.e., MeI may be unfavorably correlated with ECM, which is the denominator of the MeI ratio). In the case of feed efficiency, Kennedy et al. (1993) suggested RFI as a linearization of feed conversion efficiency (kg of milk/kg of DMI). This process was developed to avoid the use of ratio traits in genetic evaluation, as responses to selection are difficult to predict. In cases when the heritability estimates of the involved traits are similar, it is unclear which constituents of the ratio are being altered by selection pressures (Gunsett, 1984).

The argument for using residuals as a linearization of ratio traits has been deeply reviewed (van der Werf, 2004; Berry and Crowley, 2013; Hurley et al., 2017). There are both benefits and drawbacks, which are dependent on the traits being defined and the desired breeding objective. In the case of methane, a lower environmental impact that does not increase expenses or decrease profitability is required. To demonstrate this, RMP traits were compared with the ratio traits MeY and MeI.

### Defining RMP

In this research, various definitions of residual methane have been explored in an attempt to identify traits with characteristics and interactions feasible for a potential national methane breeding objective. Our research explored 9 possible definitions of RMP, all of which were found to have genetic parameters within the range of previously recorded traits of similar construct ( $h^2 = 0.1-0.3$ ; Manzanilla-Pech et al., 2016; van Engelen et al., 2018; Breider et al., 2019). Correlations between the RMP traits ( $>0.78 \pm 0.18$ ) as well as with methane candidate traits ( $>0.53 \pm 0.30$ ) were favorable; therefore, it is possible that selection for 1 methane trait may lead to indirect progress in all methane candidate traits. All RMP traits are highly genetically correlated ( $>0.85$ ), suggesting that differences between correction terms were small and that the choice of trait to be included in the breeding objective can be determined on nongenetic aspects (e.g., measurability and practicality of data collection).

Due to these circumstances,  $RMP_{pECM}$  is proposed as a selection criterion for dairy breeding programs. Although any RMP may be difficult to describe to producers,  $RMP_{pECM}$  is the most heritable of the RMP traits ( $0.21 \pm 0.10$ ) and is appealing due to its strong statistical properties and ease of inclusion in a selection

index. The  $RMP_{pECM}$  and MeI capture similar variations with a genetic correlation of  $0.86 \pm 0.09$ . The benefit of  $RMP_{pECM}$  is that it may be simply incorporated into an index. Additionally, the genetic correlation between  $RMP_{pECM}$  and MeP showed that selecting for  $RMP_{pECM}$  will reduce net emissions. The  $RMP_{pECM}$  is superior as its component traits can be easily measured in Australian dairy systems. The ECM is a phenotype that is calculated very simply from data collected routinely in dairy cows from milk production traits, which have high reliabilities (DataGene Ltd., 2018). As a candidate trait,  $RMP_{pECM}$  is a practical option to be incorporated in an index and have high reliability. The  $RMP_{pECM}$  fulfills many of the requirements to select for reduced methane emission because it has a negative, favorable genetic correlation with ECM. However, we note the correlation has a large standard error, and thus should be interpreted with caution.

The  $SF_6$  method, although laborious, allows for methane measurements to be taken on cows in a pasture setting, and data could be collected on a comparatively large number of individual cows (Moate et al., 2016). On the other hand, collecting DMI data is limited to feed trials that do not represent the commercial farm in pasture-based systems. Currently, collecting DMI records in pasture is not possible on a large number of animals, although technologies are being developed to achieve this (Seymour et al., 2019).

### Correcting for Trait Genetic Relationships

Correcting for the genetic component of DMI using FS did not result in genetic or phenotypic independence of RMP and DMI. However, phenotypically correcting for DMI resulted in an expected phenotypic correlation that was not significantly different from 0 as well as an unchanged genetic correlation. The observed correlations may be due to the use of FS in the genetic correction for feed-based traits, as DGV for DMI are unavailable in Australia. FeedSaved is an EBV for an RFI based trait, where a more negative RFI value is favorable. Although FS is an aggregate trait that considers both lifetime RFI and a maintenance requirement, thereby containing a component of DMI, it cannot fully act as the genetic adjustment for DMI. However, it may be that correcting for DGV does not produce a trait with genetic independence, and instead only increases computational complexity and error. Note that the reliability of RFI was reported to be 0.06 with a standard deviation of 0.07 by Pryce et al. (2015). A lack of genetic independence was also observed in the genotypic and phenotypic correction for ECM. The  $RMP_{pECM}$  was phenotypically, and not genetically, independent of ECM, and  $RMP_{gECM}$  was not significantly independent

of either. This finding contrasts with the findings of VanRaden et al. (2018), who obtained an RFI trait genetically and phenotypically independent from yield by using genomic predicted transmitting ability for milk net energy and BW. However, a definitive conclusion was not possible due to the large standard errors of the estimated genetic parameters in our study.

### Challenges of Small Data Sets

The small size of the data set available introduced skepticism when estimating genetic parameters and recommending a candidate trait with high confidence. Many of the parameters estimated between methane candidate traits and traits of interests (DMI and ECM) resulted in unreliable parameter estimates with high standard errors. This was a limitation of the small data set and highlights the importance of upscaling through proxy trait development, interbreed evaluations, and international collaboration. In addition to challenges with estimating genetic parameters with a small data set, the size of the data set in combination with the narrow recording windows may have caused confounding of effects in the model. Although each batch contained multiple animals, animals were not represented between batches, which may have led to confounding between batch and animals. The reason for this was to try and maximize the total number of animals with available phenotypes. Mid-infrared spectroscopy has been used to obtain large numbers of predicted methane phenotypes from routine milk samples at herd testing (Vanlierde et al., 2015); however, the viability of using these predicted phenotypes in genomic analysis has yet to be determined.

There is also the opportunity to combine beef and dairy data for genomic prediction of methane with high success in grazing animals (Khansefid et al., 2014). Previously, combining data was challenging, as traits of interest diverged to either meat or dairy production. However, methane may be isolated from this problem because it is strongly related to the breakdown of feed and the digestive capabilities of the animal, rather than the focus of energy partitioning to a specific output. More recently, Williams et al. (2019) showed that VFA proportions in ruminal fluid can be used to predict methane yield, and they suggested that their approach is among the cheapest methods for estimating methane yield of dairy cows. Thus, proxy methane phenotypes related to RMP may be able to be estimated. The most promising opportunity of increasing data set size is through large-scale international initiatives consisting of multiple stakeholders invested in the collation of high-quality feed intake and methane data. However, the variation between international partners in pheno-

type definitions, measurement techniques, and on-farm management (e.g., diet) is challenging for combining data.

## CONCLUSIONS

This research defined and compared 9 RMP candidate traits. All RMP candidate traits were highly correlated with one another as well as with other methane candidate traits (MeP, MeY, and MeI). Due to this, RMP<sub>ECM</sub> was proposed as the RMP candidate trait for inclusion in routine genetic evaluation based on its heritability and the practicality of obtaining MeP and ECM measurements on a large number of animals in a pasture-based system. However, the large standard errors associated with some estimated genetic parameters inhibited a definitive conclusion that identified an optimal RMP candidate trait for selection, highlighting the desirability for increasing the data set size. Further research investigating the response to selection of methane candidate traits and the resulting expected decrease in methane emission is required to determine the optimal methane candidate trait in dairy cattle, which may not currently be the most practical.

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# **Chapter 3:**

## **Estimating Methane Coefficients to Predict the Environmental Impact of Traits in the Australian Dairy Breeding Program**

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## Estimating methane coefficients to predict the environmental impact of traits in the Australian dairy breeding program

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### ABSTRACT

The dairy industry has been scrutinized for the environmental impact associated with rearing and maintaining cattle for dairy production. There are 3 possible opportunities to reduce emissions through genetic selection: (1) a direct methane trait, (2) a reduction in replacements, and (3) an increase in productivity. Our aim was to estimate the independent effects of traits in the Australian National Breeding Objective on the gross methane production and methane intensity (*EI*) of the Australian dairy herd of average genetic potential. Based on similar published research, the traits determined to have an effect on emissions include production, fertility, survival, health, and feed efficiency. The independent effect of each trait on the gross emissions produced per animal due to genetic improvement and change in *EI* due to genetic improvement (intensity value, *IV*) were estimated and compared. Based on an average Australian dairy herd, the gross emissions emitted per cow per year were 4,297.86 kg of carbon dioxide equivalents (CO<sub>2</sub>-eq). The annual product output, expressed in protein equivalents (protein-eq), and *EI* per cow were 339.39 kg of protein-eq and 12.67 kg of CO<sub>2</sub>-eq/kg of protein-eq, respectively. Of the traits included in the National Breeding Objective, genetic progress in survival and feed saved were consistently shown to result in a favorable environmental impact. Conversely, production traits had an unfavorable environmental impact when considering gross emissions, and favorable when considering *EI*. Fertility had minimal impact as its effects were primarily accounted for through survival. Mastitis resistance only affected *IV* coefficients and to a very limited extent. These coefficients may be used in selection indexes to apply emphasis on traits based on their environmental impact, as well as applied by governments and stakeholders to track trends in industry emissions. Although initiatives are underway

to develop breeding values to reduce methane by combining small methane data sets internationally, alternative options to reduce emissions by utilizing selection indexes should be further explored.

**Key words:** sustainability, environmental impact, methane, emission intensity, gross emissions

### INTRODUCTION

In 2016, Australia committed to reduce greenhouse gas (GHG) emissions by 26 to 28% of 2005 levels by 2030 (UNFCCC, 2016). This target relies largely on a decrease in the 3 major GHG, carbon dioxide (CO<sub>2</sub>), methane (CH<sub>4</sub>), and nitrous oxide, which account for 81%, 10%, and 7% of the global GHG inventory, respectively (EPA, 2020). For the Australian dairy sector, the primary GHG targeted to reduce its environmental impact is CH<sub>4</sub> as it accounts for 57% of the industry's emissions (UNFCCC, 2018).

Methane is associated with DMI as 90% is generated as a by-product of feed fermentation, with 6% to 11% of the energy in feed being lost to the production of CH<sub>4</sub> (Appuhamy et al., 2016). The genetic correlation between CH<sub>4</sub> and DMI has been previously estimated between 0.3 and 0.6 (Difford et al., 2020; Richardson et al., 2021b; Manzanilla-Pech et al., 2021), suggesting that the genetic architecture of these traits is similar. However, the relationship between the general selection criteria for methane emissions and feed efficiency, of which CH<sub>4</sub> and DMI are component traits, is contested in dairy cattle (Løvendahl et al., 2018; González-Recio et al., 2020). Some studies have shown that CH<sub>4</sub> per unit of feed decreases with increasing levels of DMI (Jentsch et al., 2007) and that improving feed efficiency causes a decrease in CH<sub>4</sub> (Basarab et al., 2013). However, Flay et al. (2019) reported no decrease in daily CH<sub>4</sub> with increasing feed efficiency, but did show increases in CH<sub>4</sub> per unit feed. This indicates that mitigation strategies focused on solely reducing CH<sub>4</sub> emission through genetic improvement in feed efficiency traits may not be satisfactory in lowering dairy cattle emissions, and that additional methods are required.

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Large-scale research initiatives (such as the Efficient Dairy Genome Project, 2016) are focused on developing genetic strategies to mitigate dairy cattle CH<sub>4</sub> emissions; however, EBV for CH<sub>4</sub> are not yet widely available. Measuring CH<sub>4</sub> is expensive and labor intensive, leading to small data sets. Additionally, recording protocols vary between studies, limiting the ability to combine data sets (nationally or internationally), as has been done successfully for traits with similar issues, such as DMI (de Haas et al., 2017; Hristov et al., 2018). Therefore, until reliable CH<sub>4</sub> EBV are ready for industry application, other mitigation options should be explored. Many traits, direct and proxy, have been suggested to decrease CH<sub>4</sub>. However, Wall et al. (2010) suggested that the environmental impact of livestock may also be decreased by improving individual animal productivity and efficiency, and by reducing overall waste at the herd level.

Selection indexes are traditionally comprised of traits with direct economic benefit to the producer, including production, fertility, and health. Therefore, a value must be assigned to CH<sub>4</sub> to give it a comparative weight in a selection index. In the case of CH<sub>4</sub>, defining an economic value is challenging as only marginal value directly related to improve feed efficiency exist, and many have recorded the economic value as zero (FAO, 2013). In regions where a carbon tax has been placed on agriculture, the market value of carbon may be used. However, no such tax currently exists in Australia, so this approach cannot be applied to estimate a direct value for the Australian dairy industry.

Amer et al. (2018) developed a methodology of monitoring and estimating the independent effect that index traits have on the environment for any GHG or production system. This method has since been adapted to investigate the environmental impact of the dairy and beef industry, and to estimate possible environmental index weights in beef and dairy systems internationally (Quinton et al., 2018; Zhang et al., 2019; Richardson et al., 2021a).

The aim of this research was to calculate coefficients that describe the change in CH<sub>4</sub> attributed to each index trait in the Australian dairy selection index described in gross methane and methane intensity, which may be applied as weights in a selection index to account for the environmental impact of traits.

## MATERIALS AND METHODS

The method used in this study was based on the approach used by Amer et al. (2018) and adapted to calculate the independent effects of traits in the Australian National Breeding Objective (NBO), on the gross methane production and methane intensity of an

Australian dairy herd of average genetic merit. Briefly, this method estimates the change in total emissions and product output caused by a 1-unit change in each index trait, resulting from either a direct emissions trait (methane yield), changes in herd structure (fewer replacements), or the dilution effect of higher yields (milk production) and proliferation (more offspring/dam). A primary assumption of this study is that the CH<sub>4</sub> emitted is directly proportional to the total feed consumed, as approximately 90% of the CH<sub>4</sub> emitted by cattle is produced as a by-product of feed fermentation and released as enteric CH<sub>4</sub> (Ellis et al., 2008).

### Average Australian Herd

Parameters in this study (Table 1) were assumed to be representative of the average Australian dairy herd, consisting of cows with an average 305-d production of 6,861.00 kg milk per cow at 4.00% fat, 3.30% protein and 4.80% lactose (DataGene Ltd., 2017). The average feed required per lactating cow was taken as a weighted average of the feed energy required per parity (7,207.58 kg of DM; Méndez et al., 2020). The estimated total feed required by a growing heifer (3,526.75 kg of DM) was determined based on the feed required to reach 90% of adult BW (Williams et al., 2011), the average of which was estimated to be 600 kg (Byrne et al., 2016). The energy contents of lactating cow and replacement heifer diets were assumed to be 11.91 MJ ME/kg of DM and 9.27 MJ ME/kg of DM, respectively (Williams et al., 2011; Pryce et al., 2015).

**Table 1.** Constants, conversion factors, and industry parameters based on an Australian herd of average genetic merit used to estimate base emissions, product output, changes in gross emissions due to each trait, and changes in emission intensity

Parameter	Value
Production <sup>1</sup>	
Milk yield, kg	6,861.00
Fat, %	4.00
Protein, %	3.30
Lactose, %	4.80
Feed required <sup>2</sup>	
Heifer, kg of DM	3,526.75
Cow, kg of DM	7,207.58
BW, <sup>2</sup> kg	600.00
Herd proportion <sup>1</sup>	
First parity, %	27.00
Second parity, %	22.00
Third parity, %	51.00
CH <sub>4</sub> yield, <sup>3</sup> kg/kg of DMI	19.00
CH <sub>4</sub> global warming potential <sup>4</sup>	28.00

<sup>1</sup>Values obtained from DataGene Ltd. (2017).

<sup>2</sup>Williams et al. (2011) and Pryce et al. (2015).

<sup>3</sup>Richardson et al. (2021b).

<sup>4</sup>UNFCCC (2016).

### Traits Under Investigation

The Australian national selection index, the Balanced Performance Index (**BPI**), was released in 2016. Details of the index development are given by Byrne et al. (2016). The policy in Australia is for the national selection index to be updated periodically (every 5 yr). Based on the work of Zhang et al. (2019) and Bell et al. (2013), the traits previously determined to have an impact on emissions are production traits (milk, fat, and protein), fertility, survival, health, and feed efficiency.

### Methane Coefficient for Carbon Dioxide Equivalents

The methane coefficient, defined as the environmental impact of CH<sub>4</sub> per unit of feed, was derived from the Australian methane production to DMI ratio previously calculated (19.00 g of CH<sub>4</sub>/kg of DM; Richardson et al. 2021b) and from the global warming potential (**GWP**) of CH<sub>4</sub> (28.00 kg of CH<sub>4</sub>/kg of CO<sub>2</sub>-eq; Gerber et al., 2013). The methane coefficient, expressed in CO<sub>2</sub> equivalents (**CO<sub>2</sub>e**) was determined to be 0.532 kg of CO<sub>2</sub>-eq/kg of DM (19.0 g of CH<sub>4</sub>/kg of DM × 28 g of CH<sub>4</sub>/g of CO<sub>2</sub>-eq). The relationship between methane production and DMI was previously reported to be linear (Richardson et al., 2019). Methane emissions coefficients were calculated on the basis of gross CH<sub>4</sub> per animal and CH<sub>4</sub> intensity per unit of milk protein equivalents (**protein-eq**). Both of these CH<sub>4</sub> trait definitions represent an important goal and possible future breeding objective for CH<sub>4</sub>. Defining a CH<sub>4</sub> breeding objective is specific to the reduction goals of each system and possible considerations for each CH<sub>4</sub> definition are further described in the discussion.

### Protein Conversion Factor of Product Classes

In line with previously published studies (Zhang et al., 2019; Richardson et al., 2021a), the product classes considered in this study are milk and its components (fat and protein). Similar to the concept that GWP is used to convert methane into CO<sub>2</sub> equivalents, protein-eq conversion factors,  $k_j$ , convert milk and its component traits into a single output, protein-eq. This allows for multiple product classes to be considered and evaluated, while maintaining a single product class for comparison. Protein-equivalent conversion factors were based on component values of \$6.76, \$2.08, and -\$0.11, for protein, fat, and milk, respectively (Byrne et al., 2016). The resulting conversion factors were determined to be 1.00, 0.31, and -0.02 for protein, fat, and milk, respectively. The protein-eq value for milk volume is constituted by the milk volume percentage of lactose

(4.8%) to avoid double counting the value of milk fat and milk protein, when milk volume is increased.

### Gross Methane of the Average Dairy System

Yearly gross CH<sub>4</sub> emissions produced by an average Australian dairy breeding cow, before genetic gain, were estimated using the following equation:

$$E = \sum_{i=1}^c n_i F_i \alpha Y_i, \quad [1]$$

where  $E$  is the total gross CH<sub>4</sub> emitted before genetic change per breeding cow across  $c$  classes (replacement heifers and cows),  $n_i$  is the number of animals in a given stock class  $i$  per breeding female in the average Australian dairy herd,  $\alpha$  is the methane carbon dioxide equivalent coefficient per kg of DMI (0.532 kg of CO<sub>2</sub>/kg of DM),  $F_i$  is the amount of feed (in kg of DM) per animal of the stock class  $i$ , and  $Y_i$  is a binary indicator variable that takes a value of 1 or 0 depending on whether the class  $i$  is considered in the total definition of CH<sub>4</sub> emissions, respectively. In this case, the animal classes considered were dairy cows and replacement heifers as male calves have marginal value in the Australian dairy industry.

### System Gross Emissions

Table 1 summarizes the parameters of an average Australian dairy herd, which were used to estimate  $E$ . The total gross emissions per cow, considering the average Australian dairy herd and only methane GHG emissions, is calculated as an aggregate of the emissions produced by the cow and the emissions produced by the replacement animals required on average per lactating cow to maintain the milking herd. The average herd replacement rate was defined as the proportion of first parity animals in the herd, which in Australia is currently approximately 27% (E. Ooi, Bundoora, VIC, Australia, personal communication). The feed requirement for each stock class was based on previous studies as described above (Williams et al., 2011; Pryce et al., 2015).

### Gross Emission Per Unit Change in Trait

The independent effect of each trait on the gross emissions produced per animal due to genetic improvement (**GV**) can be described by the first derivative of the above equation, with respect to a 1-unit genetic improvement in each trait, such that

$$GV = \sum_{i=1}^c \alpha Y_i \left( \frac{\partial n_i(g)}{\partial g} F_i + \frac{\partial F_i(g)}{\partial g} n_i \right), \quad [2]$$

where  $\frac{\partial n_i(g)}{\partial g}$  is the change in number of animals as a function of a 1-unit change in genetic trait  $g$ ,  $\frac{\partial F_i(g)}{\partial g}$  is the change in feed intake (kg of DM) as a function of a 1-unit change in  $g$ , and  $\alpha$ ,  $n_i$ ,  $F_i$ , and  $Y_i$  are as described above.

### Change in Gross Emission Due to Genetics

The emissions generated by a 1-unit increase in production traits (milk volume, fat, and protein) are assumed to be directly proportional to the feed required to increase each production trait by 1 unit. For fat and protein, the emissions generated were calculated as the CH<sub>4</sub> associated with the additional feed intake required (Table 2) to support an additional 1 kg of fat (6.00 kg of DM  $\times$  0.532 kg of CO<sub>2</sub>e/kg of DM = 3.19 kg of CO<sub>2</sub>e) or protein (3.70 kg of DM  $\times$  0.532 kg of CO<sub>2</sub>e/kg of DM = 1.97 kg of CO<sub>2</sub>e). When considering the total feed energy required for a 1-unit increase in milk production, only the feed required to support the additional lactose production was considered. The basis of this analysis assumes that the trait under investigation changes 1 unit, whereas all other traits are held constant. By accounting for milk production through the fluid (or lactose) portion of milk, we capture the independent effect of each production trait and avoid double counting the effects of fat and protein, as milk volume is osmotically driven by lactose. Therefore, the

emissions generated by a 1-unit increase in milk volume were 2.60 kg of DM  $\times$  0.532 kg of CO<sub>2</sub>e/kg of DM  $\times$  4.80% lactose = 0.07 kg of CO<sub>2</sub>e.

Survival has a dual effect on CH<sub>4</sub> emissions. Improvements in survival change herd structure, as higher survival rates result in fewer replacements being required to maintain the milking herd. Therefore, increased survival rates reduce the feed requirements and emissions associated with rearing replacements. However, lowering in the replacement rates will in turn increase the average age of the herd and the feed energy required for milking, as later parity cows require more feed to sustain a higher production level than the first parity counterparts (Haile-Mariam et al., 2003). The expected change in replacement rate due to a 1% increase in survival was modeled in a base herd with an average replacement rate of 27% (E. Ooi, Bundoora, VIC, Australia, personal communication) and compared with a herd where the survival rate was improved by 1%, using methods described in Zhang et al. (2019). The change in replacement rate per unit increase in survival was estimated as  $-0.0069$  (Table 3) with the associated emissions of  $-24.62$  kg of CO<sub>2</sub>e ( $-0.0069 \times 3,526.76$  kg of DM per replacement  $\times$  0.532 CO<sub>2</sub>e/kg of DM). The change in feed required per unit change in survival was modeled (Zhang et al., 2019) as the difference in feed per day required to sustain the increased milk production of a herd with 1% improved survival. The emissions associated with this increase in an average herd age was calculated as 8.55 kg of DM additional feed required  $\times$  0.532 CO<sub>2</sub>e/kg of DM = 4.55 kg of CO<sub>2</sub>e.

Much of the change in CH<sub>4</sub> due to fertility is directly related to survival, as poor fertility status is a major reason for culling (Workie et al., 2019). The increased survival rates generally observed with improved herd fertility are assumed to be largely captured by the

**Table 2.** Trait-specific effects of feed intake per stock class, number of animals in the stock class, and average feed energy produced per day

Item	$\frac{\partial n_i(g)}{\partial g}$ <sup>1</sup>		$\frac{\partial F_i(g)}{\partial g}$ <sup>2</sup>	
	Replacement	Cow	Replacement	Cow
Milk protein, kg				3.70
Milk fat, kg				6.00
Milk yield, L				0.066
Survival, %	$-0.0069$			8.55
Fertility, %				6.08
Feed saved, kg				$-1.00$
Mastitis resistance, %				0.00

<sup>1</sup>  $\frac{\partial n_i(g)}{\partial g}$  is the change in number of animals per unit change in each trait.

<sup>2</sup>  $\frac{\partial F_i(g)}{\partial g}$  is the change in the kg of feed required per unit change in each trait.

**Table 3.** Genetic trends of index traits with an effect on dairy industry emissions

Trait	5-yr annual genetic trend <sup>1</sup>
Milk protein, kg	6.5
Milk fat, kg	9.25
Milk volume, L	121.29
Survival, %	2.09
Fertility, %	0.82
Feed saved, kg	4.49
Mastitis resistance, %	0.16

<sup>1</sup>Provided by DataGene Ltd. (Bundoora).

survival EBV. However, fertility also has a direct effect on emissions. In Australia, approximately 35% of herds practice seasonal calving (Byrne et al., 2016), a system that is defined by a clearly defined mating season, which results in large numbers of cows calving in a short period of time to match peak herd energy requirements with maximum pasture availability. In this system, improved fertility results in cows calving earlier in the season and therefore having a longer lactation than cows that calve later. This in turn results in a higher feed requirement and higher emissions. The emissions produced due to a change in fertility were calculated as the additional feed required to maintain milk production during the extended lactation, where the additional production days associated with a 1% increase in fertility was modeled as 0.9985 d (Zhang et al., 2019). Therefore, the emissions associated with a 1-unit change in fertility are  $0.9985 \text{ d} \times 17.37 \text{ kg of DM} \times 35\% \text{ seasonal calving herds} \times 0.532 \text{ kg of CO}_2\text{e/kg of DM} = 3.24 \text{ kg of CO}_2\text{e}$ .

Feed saved is the Australian feed efficiency trait that consists of a lifetime residual feed intake, genomic EBV, and BW EBV (Pryce et al., 2015). Feed saved is calculated by subtracting the animal's residual feed intake from the feed required to maintain 1 kg of extra BW and expressed so that a larger value represents a more efficient animals due to the lower maintenance costs of lesser-weight cows. Animals that are either not Holstein or not genotyped have their feed saved EBV approximated by BW only. The emissions associated with a 1-unit change in feed saved is the emissions generated by the 1-unit difference in feed intake (1 kg of DM  $\times$  0.532 kg of CO<sub>2</sub>-eq/kg of DM).

It is expected that a direct CH<sub>4</sub> trait will become available in the future (Manzanilla-Pech et al., 2021; Richardson et al., 2021b). In Australia, a residual methane trait that corrects for production and intake has been developed (Richardson et al., 2021b). The emissions associated with a 1-unit increase in a residual methane trait is equivalent to the CO<sub>2</sub>e produced by a 1-unit increase in CH<sub>4</sub> (1 kg of methane  $\times$  28 GWP =

28 kg of CO<sub>2</sub>e), assuming that the trait is defined as a 1-kg decrease in residual methane per lactation.

### Emission Intensity of the Average Dairy System

The per cow environmental impact can also be expressed through emissions intensity, which is defined as the total gross emissions generated,  $E$ , divided by the total number of product output equivalents per cow ( $M$ ). In this scenario, product outputs are expressed in protein-eq and all other product classes are converted to protein-eq based on value ratios. Total product output, in protein-eq, before genetic change was calculated as follows:

$$M = \sum_{j=1}^p k_j \sum_{i=1}^c n_{ij} m_{ij} X_{ij}, \quad [3]$$

where  $M$  is the total product output, expressed in protein-eq, produced per breeding cow across all product classes,  $p$ , included in the product definition objective, before genetic change,  $n_{ij}$  is the number of animals per breeding female (specific to both stock class  $i$  and product type  $j$ ),  $m_{ij}$  is the product output per animal in product type  $j$ ,  $X_{ij}$  is the indicator variable that takes a value of either 1 or 0 depending on whether type  $j$  should be considered in the total definition of product output, and  $k_j$  is the protein equivalent conversion factor. Therefore, the emission intensity ( $EI$ ) per animal product, before genetic change, is

$$EI = \frac{E}{M} = \frac{\sum_{i=1}^c n_i F_i \alpha_i Y_i}{\sum_{j=1}^p k_j \sum_{i=1}^c n_{ij} m_{ij} X_{ij}}. \quad [4]$$

### Emission Intensity Per Unit Change in Trait

The independent effect of each trait on  $EI$  due to genetic improvement can be determined using methods adapted from Amer et al. (2018). By taking the first derivative of Equation 4 with respect to a 1-unit genetic improvement of each trait, the change in  $EI$  due to a unit change in each trait, termed intensity value ( $IV$ ), is described as

$$IV = \frac{1}{M} \left\{ \begin{array}{l} \sum_{i=1}^c \alpha_i Y_i \left( \frac{\partial n_i(g)}{\partial g} F_i + \frac{\partial F_i(g)}{\partial g} n_i \right) \\ -EI \sum_{j=1}^p k_j \left( \frac{\partial n_{ij}(g)}{\partial g} m_{ij} + \frac{\partial m_{ij}(g)}{\partial g} n_{ij} \right) \end{array} \right\}, \quad [5]$$

**Table 4.** Trait-specific change in animal product,  $j$ , as a function of change in trait,  $g$ 

Item	$\frac{\partial m_{ij}(g)}{\partial g}$ <sup>1</sup>		
	Milk protein	Milk fat	Milk yield
Milk protein, kg	1.00		
Milk fat, kg		1.00	
Milk yield, L			1.00
Survival, %	0.25	0.20	3.66
Fertility, %	0.21	0.17	4.87
Feed saved, kg			
Mastitis resistance, %	0.17	0.14	2.53

<sup>1</sup> $\frac{\partial m_{ij}(g)}{\partial g}$  is the change in product output,  $m_{ij}$  (milk, fat, and protein), generated by a unit change in each trait.

where  $\frac{\partial m_{ij}(g)}{\partial g}$  is the change in the animal product,  $j$ , per unit change in a genetic trait for stock class  $i$ , and all other variables are as previously described.

### Change in Product Output Due to Genetics

The product output for production traits (milk, fat, protein) is directly equivalent to the protein-eq for a 1-unit improvement in the production trait under investigation. Therefore, the change in product output associated with each trait is the 1-unit change in the trait converted into protein-eq (Table 4). The product output was calculated as  $-0.02$  kg of protein-eq for milk volume,  $0.31$  kg of protein-eq for fat, and  $1$  kg of protein-eq for protein.

A change in survival affects product output as a decrease in replacement rate translates to an older average herd with higher milk production, because later parity animals generally produce more than first parity animals. Therefore, the change in product output due to genetic gain in survival is the increased production expected from a herd where survival has increased by

1%. This change in product output associated with a 1-unit increase in survival was modeled as described by Zhang et al. (2019), where production of a base herd was compared with an improved-survival herd.

As survival accounts for the product output associated with changes in herd structure, the independent effect of improved fertility on product output is the additional production associated with extended lactation in seasonal calving systems. Therefore, the change in production due to fertility is the additional days of lactation due to a 1% increase in fertility ( $0.99$  d; modeled as Zhang et al., 2019)  $\times$  the proportion of Australian herds following a seasonal calving pattern (35%)  $\times$  the average daily production at the end of lactation ( $12.5$  kg of milk; Abdelsayed et al., 2015), converted into protein-eq based on the assumed percentage composition of each milk component (4.0% fat and 3.30% protein).

Mastitis resistance (**MR**) affects product output as higher MR EBV relates to lower cases of mastitis and less milk dumped due to mastitis treatment protocols. The change in product output due to MR is equivalent to the discarded milk not recovered for calf feeding ( $8.13$  kg of milk solids; Byrne et al., 2016)  $\times$  the clinical mastitis incident rate per unit change in MR ( $0.03$ ; G. Nieuwhof, Bundoora, VIC, Australia, personal communication)  $\times$  the number of treatments per incident ( $1.20$ ; Byrne et al., 2016), converted into protein equivalents based on the assumed percentage composition of each milk component (4.0% fat and 3.30% protein).

### Annual Emissions Response

To put the  $GV$  and  $IV$  coefficients into perspective, the annual expected  $CO_2e$  change in gross emissions and emissions intensity was estimated (Table 5). The  $GV$  and  $IV$  coefficients estimated for each trait were multiplied by the respective 5-yr average annual trait genetic trends (Table 2). Genetic trends were supplied by DataGene Ltd. and expected to be representative of the Australian dairy response to selection.

**Table 5.** Change in gross emissions and emissions intensity due to a unit change in each trait and expected annual change due to each trait<sup>1</sup>

Trait	$GV$ , kg of $CO_2e$ /unit trait	Expected annual change in kg of $CO_2e^2$	$IV$ , kg of $CO_2e$ /kg protein-eq	Expected annual change in $CO_2e$ /kg of protein-eq
Milk protein, kg	1.97	12.79	$-0.032$	$-0.205$
Milk fat, kg	3.19	29.52	$-0.002$	$-0.020$
Milk volume, L	0.04	4.27	0.001	0.086
Survival, %	$-8.55$	$-17.88$	$-0.035$	$-0.070$
Fertility, %	3.24	2.65	0.004	0.003
Feed saved, kg	$-0.53$	$-2.39$	$-0.002$	$-0.007$
Mastitis resistance, %	0.00	0.00	0.006	$-0.001$

<sup>1</sup> $CO_2e = CO_2$  equivalents; protein-eq = protein equivalents;  $GV$  = genetic improvement;  $IV$  = intensity value.

<sup>2</sup>Calculated using genetic trends supplied in Table 2 and provided by DataGene Ltd.

### Sensitivity Analysis

A sensitivity analysis was performed to account for the variation in product value ratio and feeding systems in Australia, which contribute to the total product output and methane coefficient, respectively. The value of fat relative to protein varies throughout and between financial years and different countries assume substantially different relative prices for fat in their national selection indexes (Miglior et al., 2017). To demonstrate the effects of this value ratio on  $M$  and  $EI$ , protein-eq conversion factors were tested at fat to protein ratios of 0.2, 0.95, and 1.25. When testing the sensitivity of modeled outcomes to the relative values of milk fat to milk protein (parameter  $k_{fat}$ ), we simultaneously manipulated the assigned value of protein to keep a constant overall milk return to the farmer and avoid artificially inflating the value of the milk components. There are 5 feeding systems actively used in Australia, each with a varying level of forage to concentrate ratio, affecting the subsequent methane coefficient (kg of CH<sub>4</sub>/kg of DMI). To account for this, a high and low methane coefficient was used to stress the system at  $\pm 5\%$ ,  $10\%$ , and  $20\%$ .

## RESULTS

### System Emissions Without Genetic Change

Based on an average Australian dairy herd, the gross emissions emitted per breeding cow were 4,297.86 kg of CO<sub>2</sub>-eq per year. The annual product output and emissions intensity per breeding cow were 339.29 kg of protein-eq and 12.67 kg of CO<sub>2</sub>-eq/kg of protein-eq, respectively.

### Change in Gross Emissions Attributed to Genetics

Results are presented in Table 5, with values for intermediate calculations for changes in herd structure and feed requirements presented in Table 3. When considering the change in gross emission associated with a unit change in each trait, survival ( $-8.55$  kg of CO<sub>2</sub>-eq) had the most favorable  $GV$  with feed saved ( $-0.53$  CO<sub>2</sub>-eq) being the only other trait that lowered emissions. Milk, fat, and protein had unfavorable  $GV$  of 0.04, 3.19, and 1.97 kg of CO<sub>2</sub>-eq, respectively, and fertility had the largest unfavorable effect of 3.24 kg of CO<sub>2</sub>-eq kg of CO<sub>2</sub>-eq.

To estimate the expected change in emissions related to each trait relevant to the expected response to selection, each  $GV$  was multiplied by its respective 5-yr annual genetic trend (Table 2). Under this perspective (Table 5), survival and feed saved had the most favor-

able environmental impacts ( $-17.88$  and  $-2.39$  kg of CO<sub>2</sub>-eq/year, respectively). Fertility, milk volume, and milk protein had unfavorable environmental impacts of 2.65, 4.27, and 12.79 kg of CO<sub>2</sub>-eq/year. The largest unfavorable annual change in emissions was due to milk fat at 29.53 kg of CO<sub>2</sub>-eq/year.

### Change in Emissions Intensity Attributed to Genetics

Results are presented in Table 5, with values for intermediate calculations for changes in gross emissions and product outputs presented in Tables 3 and 4, respectively. Without considering the magnitude of the annual genetic improvement of each trait, survival ( $-0.034$  kg of CO<sub>2</sub>-eq/kg of protein-eq) had the most favorable EV. Milk protein ( $-0.032$  CO<sub>2</sub>-eq/kg of protein-eq) had the second largest favorable effect with MR having a moderate favorable effect of  $-0.006$  CO<sub>2</sub>-eq/kg of protein-eq. Fat and feed saved also lowering emissions intensity, both with the smallest favorable EV of  $-0.002$  CO<sub>2</sub>-eq/kg of protein-eq (Table 5). Contrastingly, fertility and milk yield had unfavorable effects of 0.004 and 0.001 CO<sub>2</sub>-eq/kg of protein-eq, respectively.

Multiplying the 5-yr annual genetic trend by each trait  $EI$  value, the expected annual change in emissions intensity related to each trait was presented in Table 5. Under this perspective, milk protein and survival had the most favorable environmental impacts ( $-0.21$  and  $-0.07$  kg of CO<sub>2</sub>-eq/kg of protein-eq per year, respectively). Additionally, milk fat and feed saved had favorable annual reductions in  $EI$  of  $-0.02$  and  $-0.007$  CO<sub>2</sub>-eq/kg of protein-eq per year, respectively. Fertility and MR had very minimal effects on  $EI$  with annual  $IV$  of 0.002 and 0.001 kg of CO<sub>2</sub>-eq/kg of protein-eq per year, respectively.

### Sensitivity Analysis

The effects of changing fat to protein ratio values as well as variations in methane coefficients on  $E$ ,  $M$ , and  $EI$  are presented in Table 6. The  $EI$  calculated under the protein-eq conversion ratios, which represent the 2-extreme scenarios (0.20 and 1.25), were 12.50 CO<sub>2</sub>-eq/kg of protein-eq per year and 10.03 CO<sub>2</sub>-eq/kg of protein-eq per year, respectively. However, the emissions per kg of protein-eq compared with the base estimation of  $EI$  (12.67 CO<sub>2</sub>-eq/kg of protein-eq per year) varied by only 0.0025% when the protein-eq conversion ratio was increased to 0.95, in agreement with the current global trend. As the fat to protein value ratio approaches 1.00, the value of fat more becomes more equivalent to protein and the  $IV$  for fertility becomes negligible due to the increased product output being realized. The change in  $E$  and  $EI$  was directly

**Table 6.** Sensitivity analysis accounting for the variation in product value ratio and feeding systems in Australia, where protein-equivalent conversion factors were stressed at a fat to protein value ratio ( $k_{fat}$ ) of 0.2, 0.95, and 1.25, and a high and low methane coefficient was used to stress the system at  $\pm 5\%$ , 10%, and 20%

Item	Base ( $k_{fat} = 0.31$ )	$k_{fat} = 0.20$	$k_{fat} = 0.95$	$k_{fat} = 1.25$
$E^1$	4,297.86	4,297.86	4,297.86	4,297.86
$M^2$	339.29	343.75	385.40	428.62
$EI^3$	12.67	12.50	11.15	10.03
	Base	+5%	+10%	+20%
$E$	4,297.86	4,512.76	4,964.03	5,157.44
$M$	339.29	339.29	339.29	339.29
$EI$	12.67	13.30	14.63	15.20
	Base	-5%	-10%	-20%
$E$	4,297.86	4,082.97	3,868.08	3,438.29
$M$	339.29	339.29	339.29	339.29
$EI$	12.67	12.03	11.40	10.13

<sup>1</sup> $E$  is the total gross methane emitted before genetic change per breeding.

<sup>2</sup> $M$  is the total product output, expressed in protein-equivalents, produced per breeding.

<sup>3</sup> $EI$  is the emission intensity before genetic change.

proportional to the  $\pm 5\%$ , 10%, or 20% change in the methane coefficient when compared with the base.

## DISCUSSION

The aim of this research was to calculate coefficients that describe the change in methane attributed to traits under selection in Australian dairy cattle in gross methane and methane intensity. Our results estimate gross emissions per breeding cow of 4,297.86 kg and an emissions intensity of 12.67 kg of  $\text{CH}_4/\text{kg}$  of protein-eq, and consistently showed the importance of selection for survival, production, and efficiency through the estimated coefficients. Using the gross methane per breeding cow value estimated in our study, we estimated a national inventory due to dairy cattle methane of 6.02 Mt of  $\text{CO}_2\text{e}$ , assuming that the Australian dairy cattle inventory was 1.4 million milking and dry cows (Newton et al., 2020). These values are reasonably consistent with the current Australian national emissions inventory for the dairy industry of 8.6224 Mt of  $\text{CO}_2\text{e}$  (UNFCCC, 2018), of which methane constitutes approximately 57% at 4.914 Mt of  $\text{CO}_2\text{e}$ .

### Indirect Methods to Reduce Emissions

The BPI is the national dairy cow selection index in Australia (Byrne et al., 2016) and includes traits that contribute to profitability including production, survival, fertility, MR, and feed efficiency. Currently the index does not include methane directly, but it does include traits such as longevity and feed saved that, as we have shown in the present study, can have a favorable effect on methane emissions.

As suggested by Wall et al. (2010), there are 3 possible opportunities to reduce emissions per unit of prod-

uct through genetic selection: (1) a direct  $\text{CH}_4$  trait, (2) a reduction in replacements, and (3) an increase in product output per animal. However, opportunities 1 and 2 may also be applied to reduce gross emissions. In this paper we explored options that did not require a direct  $\text{CH}_4$  trait, but rather relied on existing EBV. Consistent with Wall et al. (2010), our results imply that reducing replacements through selection for longevity and fertility and increasing production efficiency through feed saved may be effective strategies to reduce gross emissions and emissions intensity. Additionally, the dilution effect of product output achieved through gains in production traits and MR may also be effective in reducing emissions intensity. However, unless the increase in production output results in fewer overall animals, such as is the case with supply management (Richardson et al., 2021a), the effect may not be environmentally beneficial. As the rate of genetic gain in production traits is increasing (Cole et al., 2020), so is the feed requirement and gross emissions. This trend is likely to mean that while  $\text{CH}_4$  intensity is decreasing, gross emissions are increasing, consistent with the unfavorable gross emissions environmental weights for production traits estimated in our study.

### Independent Trait Effects

Of the traits included in the Australian NBO, genetic progress in survival and feed saved were consistently shown to result in a favorable environmental impact. Conversely, production traits had an unfavorable environmental impact when considering gross emissions, and favorable when considering emissions intensity. These conflicting results were expected as genetic gain in production traits will lead to high yielding cows with increased feed requirement, resulting in higher

gross emissions and an unfavorable environmental impact. However, when methane intensity is considered, the negative effect of increased feed requirement is overbalanced by the product output of milk and its components, resulting in a favorable environmental impact when the number of animals is reduced. A key assumption of the present study is that the independent effect of each trait is estimated, while all other traits remain static, to avoid double counting. For example, if changes in milk production were to be accounted for through ECM, then we would be assuming that fat and protein are increasing proportionally to the milk BV, contrasting to our model assumptions and resulting in double counting.

The challenge of calculating the independent effect of each trait, when the change in emissions cannot be isolated to a single trait, is also observed in traits with an effect on herd structure. Survival and fertility are intertwined at the management level, creating challenges when calculating the effect of each trait independently. Improved fertility at the herd level has a positive effect on the environment. However, this is primarily due to higher survival rates and the requirement of fewer heifers, which is captured through the survival BV. In the case of fertility and survival, a 1-unit increase in either trait results in a reduction of emissions through a change in herd structure, as fewer replacements are required. Additionally, in the case of emissions intensity, an older herd will have a higher production level, and therefore lower emissions intensity. As fertility is a primary reason for culling, the effects of the 2 traits are confounded (Workie et al., 2019). To avoid double counting, we attributed these effects solely to survival. This confounding factor may be overcome by using a survival trait that accounts for voluntary culling, such as herd life (Richardson et al., 2021a) or residual survival (Zhang et al., 2019). This is shown in New Zealand where the survival is represented through residual survival, a survival trait that is independent of fertility. In Zhang et al. (2019), the positive impacts of replacement reductions could be attributed to each trait separately.

### Sensitivity Analysis

Similar to Zhang et al. (2019), our results showed that varying the protein to fat value ratio influences *EI* through the protein-eq value and concluded that increasing the protein-eq value ratio reduced *EI* through increasing *M*. Thus, changing the protein-eq value changes the magnitude of the milk protein equivalent units, but not the overall trend in *EI* due to genetic gain. The exception to this is fertility as the effect of the fertility *IV* on *EI* became negligible with more

equivalent values of fat to protein. This change is due to the trait definition of fertility used in the current study. As the change in herd structure due to fertility is accounted for by the survival EBV, only the change in production due to extended lactation is captured by fertility. Therefore, the fertility *IV* is affected by the change in the protein-eq ratio value. However, the overall effect of the fertility *IV* on *EI* is minimal and therefore does not strongly affect the overall trend.

### Comparison to International Studies

Using adapted methods of the approach proposed by Amer et al. (2018), production, survival, and fertility were identified as key traits to reduce emissions in the New Zealand dairy (Zhang et al., 2019), Canadian dairy (Richardson et al., 2021a), and Irish dairy and beef (Amer et al., 2018; Quinton et al., 2018) industries. These industry cases differ in breeding objective, feeding system, housing system, and product output; however, the traits identified as having the largest environmental impact were consistent.

Bell et al. (2013) used a bio-economic model to investigate the environmental impact of Australian dairy traits. While they included nitrogen output and manure management in addition to methane, our results are remarkably similar. It is noteworthy that the weights developed in our study account for relationships between traits and therefore could be applied to EBV to estimate methane emissions, whereas those of Bell et al. (2013) were not designed to do this. This is particularly evident in the fertility and survival values, which is further discussed below.

### Application of Gross and Intensity Weights

Genetics is an option that offers a permanent solution to reduce emissions and there are several strategies that can be simplistically incorporated in breeding programs to target a reduction in emissions, one of which is the application of environmental weights. Gross methane weights and methane intensity weights are 2 definitions of emission coefficients that quantify the effect of current traits on the environment. These weights may be used in selection indexes to apply emphasis on traits based on their environmental impact.

In terms of breeding objectives, gross CH<sub>4</sub> production is currently the metric used by the Intergovernmental Panel on Climate Change in the Paris Agreement to estimate and report national GHG inventories on a global scale. Using gross CH<sub>4</sub> coefficients in selection indexes allows for the changes in methane achieved through genetic selection to be clearly conceptualized by many stakeholders, such as researchers, dairy producers, and

government. Gross CH<sub>4</sub> is a linear trait definition with a conceptually clear response to selection as it represents the direct, independent change in methane associated with each index trait. However, considering gross emissions does not take into consideration that for some industries, such as the dairy industry, we require the industry to continue to grow, thereby increasing the challenge to reduce emissions at an industry level.

Methane intensity coefficients are designed to estimate the environmental productivity of traits, when considering both product output and the environment. This EBV has the benefit of ensuring the industry remains sustainably productive, leaning toward a more net neutral system. However, methane intensity is a ratio trait and the response to selection associated with its component traits (gross emissions and product output) are more challenging to attribute. It is unclear whether a reduction in methane intensity due to genetic improvements is from a decrease in gross emissions or a dilution effect through increases in production, which unless animal numbers decrease as a result (such as with supply management in Canada), is not environmentally advantageous.

The development of future indexes should consider the possible environmental responsibilities of farmers. A major factor for continual genetic improvement is the index adoption rate by farmers. Methane intensity coefficients may be more appealing to farmers as the estimated coefficients do not penalize high (solids) producing cows; however, if the industry is required to reduce net emissions, a gross methane coefficient may be more favorable as genetic progress is conceptually clear and a direct reduction is simple to report.

### **Inventory Application of Methane Coefficients**

The results from this study can be used by geneticists and stakeholders. For geneticists, the weights could be used to develop indexes that farmers can apply to select for reduced methane emissions, whereas for other stakeholders, such as government, the weights could be used to capture the genetic and phenotypic changes in methane emissions over time, offering an additional use for these weights. Due to the limitation of methane data collection on-farm, it is challenging to estimate emissions at the farm or individual animal level. These coefficients can be applied to the EBV of an individual animal or the farm average genetic gain to more accurately estimate current GHG inventories, as more of the variation between animals is captured compared with current Intergovernmental Panel on Climate Change methods. This method may also be applied to other GHG or product outputs to calculate coefficient that would allow for a more robust inventory estimate to be

made. This may assist stakeholders or governments to trace farm-specific methane emissions, instead of using simplistic methods that count the number of cows, and assume constant emissions per cow both across farms, and over time. The method used in our study requires minimal inputs and less computational complexity to estimate a cow-specific emissions value and may improve long-term tracing of changes in dairy industry methane emissions.

### **Future Strategies**

This method offers one strategy to reduce emissions; another is by selecting for a direct CH<sub>4</sub> trait. Many studies have aimed to define the optimal CH<sub>4</sub> trait to select for lower emissions. However, the trait definitions currently proposed for inclusion in a breeding program are not adequate as they are derived from small data sets and challenging to predict accurately. Previous studies aimed at predicting EBV for expensive or difficult to measure traits, such as DMI (Berry et al., 2014), have successfully increased data set size by combining data sets internationally. Methane introduces new challenges when implementing this approach as the analysis relies on many small data sets using different measurement techniques, introducing additional error. As methane for each country must be considered an independent trait, multitrait models are used which increases computational complexity (Manzanilla-Pech, 2021).

It is expected that a direct CH<sub>4</sub> EBV may become available in the near future (Manzanilla-Pech et al., 2021; Richardson et al., 2021b). In Australia, a residual methane trait that corrects for production and intake is most likely to be implemented (Richardson et al., 2021b). However, due to the limitation of data ( $n = 420$ , Richardson et al., 2021a) and challenges associated with combining data sets (Manzanilla-Pech et al., 2021), it is expected that the accuracy of genomic predictions will be low (González-Recio et al., 2020). Therefore, the optimal method to reduce emissions may be to develop a subindex comprised of a direct methane trait and the traits shown to have the largest effect on emissions, weighted using the coefficients estimated in the current study.

### **CONCLUSIONS**

This paper describes estimated methane coefficients that describe the expected change in methane per unit change in each trait, defined in gross emission and emissions intensity. Of the traits included in the Australian NBO, genetic progress in survival and feed saved were consistently shown to result in a favorable environmental impact. Conversely, production traits had an unfa-

avorable environmental impact when considering gross emissions, and favorable when considering emissions intensity. Fertility had minimal effect as its effects were primarily accounted for through survival, with MR only affecting *EI* coefficients. These coefficients may be used in selection indexes to apply emphasis on traits based on their environmental impact, as well as applied by government and stakeholders to track trends in industry emissions. Although current initiatives are underway to develop EBV for methane by combining small methane data sets internationally, they are hampered by small numbers and different ways of measuring methane. Therefore, alternative options to reduce emissions by utilizing selection indexes should be further explored.

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# **Chapter 4:**

## **A Method for Implementing Methane Breeding Values in Australian Dairy Cattle**

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# A method for implementing methane breeding values in Australian dairy cattle

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## Abstract

**Context.** There has been a lot of interest in recent years in developing estimated breeding values (EBVs) to reduce methane emissions from the livestock sector. However, while a major limitation is the availability of high-quality methane phenotypes measured on individual animals required to develop these EBVs, it has been recognised that selecting for improved efficiency of milk production, longevity, feed efficiency and fertility may be an effective strategy to genetically reduce methane emissions in dairy cows.

**Aim.** Applying carbon dioxide equivalents (CO<sub>2</sub>-eq) weights to these EBVs, we hypothesise that it is possible to develop a genetic tool to reduce greenhouse-gas emissions (GHG).

**Methods.** We calculated the effect of an EBV unit change in each trait in the Balanced Performance Index on CO<sub>2</sub>-eq emissions per cow per year. The estimated environmental weights were used to calculate a prototype index of CO<sub>2</sub>-eq emissions. The final set of EBVs selected for inclusion in the GHG subindex were milk volume, fat yield and protein yield, survival and feed saved, as these traits had an independent effect on emissions. Feed saved is the Australian feed efficiency trait. A further modification was to include a direct methane trait in the GHG subindex, which is a more direct genomic evaluation of methane estimated from measured methane data, calculated as the difference between actual and predicted emissions, for example, a residual methane EBV.

**Key results.** The accuracy of the GHG subindex (excluding residual methane EBV) is ~0.50, calculated as the correlation between the index and gross methane (using 3-day mean gross methane phenotypes corrected for fixed effects, such as batch and parity and adjusting for the heritability). The addition of the residual methane EBV had a minimal effect with a correlation of 0.99 between the indexes. This was likely to be due to limited availability of methane phenotypes, resulting in residual methane EBVs with low reliabilities.

**Conclusions.** We expect that as more methane data becomes available and the accuracy of the residual methane trait increases, the two GHG subindexes will become differentiated. When the GHG subindex estimates are applied to bull EBVs, it can be seen that selecting for bulls that are low emitters of GHG can be achieved with a small compromise in the BPI of ~20 BPI units (standard deviation of BPI = 100).

**Implications.** Therefore, selection for more sustainable dairy cattle, both economic and environmental, may be promptly implemented until sufficient data are collected on methane.

**Keywords:** methane emission, sustainability, selection index, index weights.

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## Introduction

It is recognised that within dairy systems, the largest source of greenhouse gas (GHG) emissions is from enteric fermentation and methane (CH<sub>4</sub>) production (g/day). Over 30 years (between 1980 and 2010), Moate *et al.* (2016) estimated that production of enteric CH<sub>4</sub> has been almost static (185 000 t in 1980 versus 182 000 t in 2010). At the same time, milk production has increased, so that the intensity of CH<sub>4</sub> emissions (i.e. emissions per unit of product, in this case, milk) has declined considerably by 40%, from ~33.6 g CH<sub>4</sub>/kg

milk to 19.9 g CH<sub>4</sub>/kg milk. Since milk production traits are a large part of the breeding goal, it follows that the reduction in GHG has been, in part, the result of genetic improvement in efficiency and dilution of emissions per litre of milk produced.

The Australian national breeding objective, known as the Balanced Performance Index (BPI), includes traits that contribute to cow profitability, farmer preferences and desired gains (Byrne *et al.* 2016). The BPI encompasses biological traits associated with milk production, longevity, fertility, feed efficiency and health. Feed-efficiency breeding

values, known as feed saved, include genomic breeding values for residual feed intake and breeding values for maintenance requirements based on predicted cow bodyweight (Pryce *et al.* 2015). Thus, by selecting for production traits and feed saved simultaneously, it is anticipated that gross efficiency will improve in Australian dairy cows (Byrne *et al.* 2016).

There has been a lot of interest in recent years in developing estimated breeding values (EBVs) to reduce GHG from the livestock sector. However, a major limitation has been the quantity of high-quality CH<sub>4</sub> phenotypes measured on individual cows required to develop these EBVs. Currently, most published reliabilities are <10% (Manzanilla-Pech *et al.* 2021), which is insufficient for implementation of genomic prediction. However, it has been recognised that selecting for improved efficiency of milk production, longevity and fertility has had a beneficial effect on GHG emissions (Løvendahl *et al.* 2018). Consequently, it might be possible to develop an index that includes the contribution of these traits to GHG emissions, either separately, or in addition to CH<sub>4</sub> emissions. Breeders with an interest in selecting for reduced GHG emissions, without greatly affecting profitability, can select for high-BPI and low-GHG emitters.

An approach that could be suitable is to estimate the increment in CO<sub>2</sub>-eq per unit change in EBVs that are current selection objectives, while accounting for interactions among traits, thereby calculating trait-specific environmental coefficients that are independent, such as those in Amer *et al.* (2018) and Richardson *et al.* (2021a) and that, for selection index purposes, are superior to non-independent coefficients, such as those calculated by Bell *et al.* (2013) and Pryce and Bell (2017).

The aim of this research was to (1) develop a GHG subindex using the gross CO<sub>2</sub>-eq values derived by Richardson *et al.* (2021a) as subindex weights for the EBVs most strongly associated with GHG emissions (milk, fat and protein yield, longevity, and feed saved), (2) modify the GHG subindex to include residual CH<sub>4</sub> EBVs, as defined by Richardson *et al.* (2021b), from measured CH<sub>4</sub> data phenotypically corrected for energy-corrected milk and (3) validate the GHG subindex with CH<sub>4</sub> phenotypes corrected for fixed effects. Finally, we explored the effect of current selection objectives on CH<sub>4</sub> by regressing the derived subindexes for GHG on BPI and Health Weighted Index (HWI).

## Materials and methods

Previously calculated CH<sub>4</sub> coefficients (kg CO<sub>2</sub>-eq/unit change in trait) were used as weights and applied to EBVs commonly used in selection and most strongly associated with emissions to derive two possible subindexes aimed to rank the environmental impact of individual animal on the basis of their genetic merit. The first index includes traits that are currently included in the national breeding objective and known to have a direct environmental impact, including production traits, survival and feed efficiency. The second includes these index traits as well as a direct CH<sub>4</sub> trait. The two subindexes were correlated with current national breeding indexes (BPI and HWI), as well as additional traits of interest.

### *Australian national breeding objective*

Currently, there are two main indexes used for ranking dairy cattle in Australia. The BPI includes traits pertaining to production (milk, fat, protein), fertility, survival, health and feed efficiency. The HWI, which was developed using the same traits as in the BPI, places additional emphasis on traits related to health and efficiency, such as fertility and feed saved. Details of the index development are given in Byrne *et al.* (2016). DataGene (Melbourne, Vic., Australia) calculates the BPI and HWI routinely for all cows and bulls included in national genetic evaluations.

### *Phenotypic and genetic cow data*

In total, 1712 individual cow CH<sub>4</sub> measurements were obtained from 464 cows measured over a 5-day period from 12 experimental batches across 5 years from 2013 to 2017. Cows were located at the Ellinbank SmartFarm (Melbourne, Vic., Australia) and milked twice per day. Measurements for CH<sub>4</sub> (g/day) were performed using the SF<sub>6</sub> tracer method previously described by Deighton *et al.* (2014). Records for CH<sub>4</sub> were averaged to obtain one observation per animal to account for day-to-day variation (Moate *et al.* 2016). Animals were between 68 and 187 days in milk at the start of measurement and across parities 1–9. Genotypes for the 464 cows used in this study were provided by DataGene Ltd. After editing by excluding genotype calls with a GenTrain score <0.6 using the methods described by Erbe *et al.* (2012), 47 162 single nucleotide polymorphism (SNP) markers were available for genomic analysis. EBVs for the 464 animals used in this study were estimated as part of the routine genetic evaluation service of DataGene Ltd and included milk, fat, protein, survival and feed saved, which are traits directly used in GHG subindex development.

### *Genetic bull data*

Genotypes for the 3412 registered Holstein bulls used in this study were provided by DataGene Ltd, with processing and genotyping methods being consistent with the cow dataset. Bulls were born between 2010 and 2015. The EBVs used in this analysis included milk volume, milk fat, milk protein, survival and feed saved, as well as other traits of interest such as heat tolerance and liveweight and were accessed from the April 2020 official genetic evaluation run.

### *Residual CH<sub>4</sub> breeding value*

Richardson *et al.* (2021b) investigated nine definitions of residual CH<sub>4</sub> traits and concluded that CH<sub>4</sub> production corrected for energy-corrected milk is an appropriate selection candidate to reduce environmental impact without severely affecting other traits, such as production, health and fertility. Using this definition of residual CH<sub>4</sub> EBVs, we calculated genomic breeding values for bulls (or cows) that were not part of the reference dataset, by multiplying the vector of SNP effects with the genotype matrix of bulls. These SNP effects were derived using a process to back-calculate SNP effects from direct genomic values (DGVs) of cows in the reference population. In brief, using DGVs of CH<sub>4</sub> production corrected

for energy-corrected milk of 464 Ellinbank cow reference population, 41 276 SNP effects were estimated as:

$$\hat{\beta} = \mu + Z'(ZZ')^{-1}\hat{g},$$

where  $Z$  is the genotype matrix (464 individuals  $\times$  41276 SNP), and  $\hat{g}$  a vector of descaled DGVs with a mean of 0 estimated using genomic best linear unbiased prediction (VanRaden 2008). The prediction equations of SNP effects were then used to predict residual CH<sub>4</sub> DGVs of the 3412 Holstein bulls.

#### Methane coefficients

Methane coefficients were previously calculated by Richardson *et al.* (2021a) on the basis of the approach used by Amer *et al.* (2018) and adapted to calculate the effect of a unit change in milk, fat, protein, feed saved, and survival traits on CO<sub>2</sub>-eq emissions per cow and per kilogram of protein equivalents. Protein equivalents are a weighted aggregate of the product outputs from milk protein, milk fat, and milk volume weighted on the component value ratio relative to protein. Briefly, this method estimates the change in total emissions and product output caused by a 1 unit change in each index trait, resulting from either a direct emissions trait (CH<sub>4</sub> yield), changes in herd structure (fewer replacements), or the dilution effect of higher yields (milk production) and proliferation (more offspring/dam). The traits used in the current study were determined to have an independent effect on emissions, with the addition of fertility (Richardson *et al.* 2021a). However, as fertility is a primary reason for culling, the environmental impact of fertility is largely accounted for by the survival EBV, with minimal additional effects applying to extended lactations observed in seasonal calving systems (Workie *et al.* 2019; Richardson *et al.* 2021a). Therefore, only the survival CH<sub>4</sub> coefficient is considered in the index. The model was used in the current study to dynamically represent an Australian dairy herd and assess effects of changes in traits. The effects of a unit change of a trait on GHG emissions are shown in Table 1. For example, a unit change in milk protein is estimated to be associated with a GHG emission of 1.97 kg of CO<sub>2</sub>-eq (3.70 kg DM/kg protein yield  $\times$  0.532 kg CO<sub>2</sub>eq / kg DM = 1.97 kg CO<sub>2</sub>eq/kg protein yield).

**Table 1. Responses in carbon dioxide equivalents (CO<sub>2</sub>-eq) per unit change of key traits under selection**

Values are means and standard deviations ( $\sigma$ ) of breeding values in the bull population

Parameter	Mean	$\sigma$	Response (kg CO <sub>2</sub> -eq/unit change of trait) <sup>A</sup>
Milk protein (kg)	12.94	7.30	1.97
Milk fat (kg)	10.21	11.30	3.19
Milk volume (L)	319.23	340.91	0.04
Survival (%)	104.01	3.32	-10.19
Feed saved (kg)	-3.12	75.78	-0.53

<sup>A</sup>Richardson *et al.* (2021a).

#### GHG index

Indexes for CO<sub>2</sub>-eq emissions per cow per year were calculated by multiplying the EBVs of each trait in the breeding objective by the CH<sub>4</sub> coefficient (i.e. effect of a single unit change of the trait on CO<sub>2</sub>-eq emissions). The sum of this is the total CO<sub>2</sub>-eq index. EBVs selected for this study included milk, fat, protein, feed saved and survival (Amer *et al.* 2018; Richardson *et al.* 2021a). Gross CH<sub>4</sub> coefficients were applied to trait EBVs to calculate the GHG index as follows:

$$GHG_{index} = \sum_{i=1}^i w_i * EBV_i$$

where  $w_i$  is the CH<sub>4</sub> coefficient (gross or intensity) of the  $i$ th trait and  $EBV_i$  is the estimated breeding value of the  $i$ th trait (milk, fat, protein, feed saved or fertility).

#### GHG index with residual CH<sub>4</sub> production

An additional index was developed that extends the GHG index described above through the addition of the EBV of residual CH<sub>4</sub> and was calculated as follows:

$$GHG_{index}^+ = GHG_{index} + EBV_{RMP}$$

where  $EBV_{RMP}$  is the residual CH<sub>4</sub> EBV and  $GHG_{index}$  is described above.

#### Correlations

The GHG indexes were correlated with the Australian national selection indexes, as well as with index traits currently estimated through the national genetic evaluation services (DataGene Ltd). Correlations were estimated using the `cor.test()` function in R.4.0.4 statistical programming (R Core Team 2013).

#### Validation of GHG index using CH<sub>4</sub> phenotypes

The GHG<sub>index</sub> subindex was validated using a 12-fold cross-validation, where the correlation between GHG<sub>index</sub> and CH<sub>4</sub> phenotypes for each experimental batch was independently estimated, that is, batch by batch. EBVs for index traits were provided by DataGene Ltd for milk, fat, protein, survival and feed saved on the 464 Ellinbank cows used in developing the residual CH<sub>4</sub> trait. For the 464 cows, first GHG<sub>index</sub> was calculated and then the values were correlated with SF<sub>6</sub> CH<sub>4</sub> phenotypes. As described by Su *et al.* (2012), to calculate the predicted accuracies of the true breeding values, instead of the EBVs, the correlations between the GHG subindex and SF<sub>6</sub> CH<sub>4</sub> phenotypes was divided by the square root of the heritability of residual CH<sub>4</sub> production ( $h^2 = 0.21$ ; Richardson *et al.* 2021b).

The size of the dataset was insufficient to conduct a validation analysis for the GHG<sub>index</sub><sup>+</sup>. This is because all data on 464 Ellinbank cows were required for development of the genomic prediction equation to estimate  $EBV_{RMP}$ , so an independent dataset was not available.

## Results

#### GHG index values

The GHG<sub>index</sub> subindex was applied to 3412 Holstein bulls and had a mean value of 38.22 kg CO<sub>2</sub>-eq, with a standard

deviation of 76.39. When residual CH<sub>4</sub> was incorporated into the subindex, GHG<sup>+</sup><sub>index</sub>, the standard deviation was 76.62 with a mean of 37.65 kg CO<sub>2</sub>-eq.

#### Correlation between indexes

Correlations between the GHG subindexes, BPI and HWI are presented in Table 2. The two GHG-based indexes, with and without the RMP EBV, were highly correlated (0.99). The BPI was uncorrelated with the GHG<sub>index</sub> subindex and GHG<sup>+</sup><sub>index</sub> subindex, with correlations of close to zero at -0.03 and -0.02 respectively. Favourable correlations were estimated between HWI and both GHG-based subindexes, with correlations of -0.35 with GHG<sub>index</sub> and -0.36 with GHG<sup>+</sup><sub>index</sub>. These trends were consistent within the top 300 BPI bulls, where the average BPI of the top 30 BPI bulls was 333. Considering only bulls whose GHG index value was below zero, the average BPI for the top 30 BPI bulls decreased to 312, representing a low 21-point difference (Fig. 1).

#### Correlation with index traits

Correlations between the GHG indexes and selected EBVs are presented in Table 2. The GHG<sub>index</sub> and GHG<sup>+</sup><sub>index</sub> subindexes had strong favourable correlations to feed saved (-0.71 and -0.70 respectively), fertility (-0.52 and -0.51) and survival (-0.43 and -0.42), as well as to heat tolerance (-0.45 and -0.46). Low to moderate favourable correlations were observed between the indexes and mastitis resistance (-0.29 and -0.28) and RMP (0.01 and 0.07). The traits with the largest unfavourable correlation with both indexes were fat (0.66), protein (0.57) and liveweight (0.56). Milk, milking temperament, likability and overall type had low to moderately unfavourable correlations of 0.30, 0.17, 0.16 and 0.14 respectively.

**Table 2.** Correlation between GHG indexes and BPI, HWI and other EBVs within the population of 3412 registered Holstein bulls

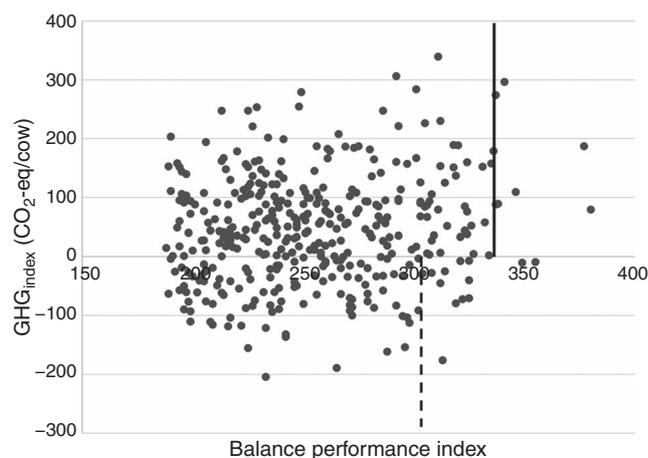
Trait	GHG index	s.e.	GHG index + RMP	s.e.
	<i>Favourable</i>			
BPI	-0.02	0.02	-0.02	0.02
HWI	-0.36	0.02	-0.33	0.02
RMP (kg CH <sub>4</sub> )	0.01	0.02	0.08	0.02
Feed saved (kg DM)	-0.71	0.01	-0.70	0.01
Fertility (%)	-0.52	0.02	-0.51	0.02
Calving ease	-0.13	0.02	-0.13	0.02
Gestation length	-0.04	0.02	-0.04	0.02
Survival (%)	-0.43	0.01	-0.42	0.01
Mastitis resistance (%)	-0.29	0.02	-0.28	0.02
SCC	-0.43	0.02	-0.28	0.02
Heat tolerance	-0.45	0.02	-0.46	0.02
	<i>Unfavourable</i>			
Milk (L)	0.30	0.02	0.31	0.02
Protein (kg)	0.57	0.02	0.57	0.02
Fat (kg)	0.66	0.01	0.66	0.01
Liveweight (kg)	0.56	0.02	0.56	0.02
Milking speed	0.07	0.02	0.06	0.02
Milking temperament	0.17	0.02	0.17	0.02
Likability	0.16	0.02	0.15	0.02
Overall type	0.14	0.02	0.13	0.02

#### Validation

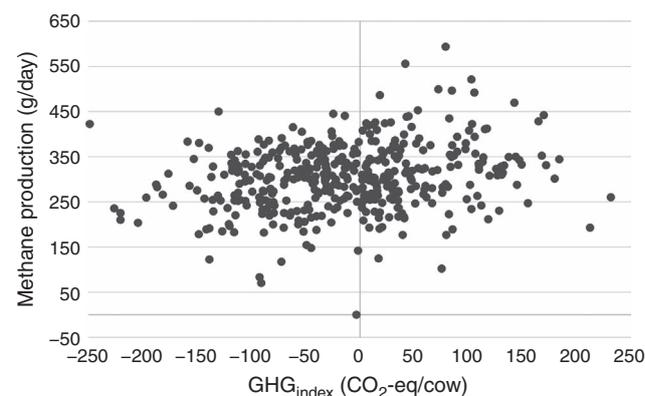
When GHG index values were correlated with SF<sub>6</sub> CH<sub>4</sub> data (Fig. 2, Table 2), the average correlation of the 12 validation batches was 0.23 (ranging 0.07–0.41). This gives the GHG<sub>index</sub> subindex an accuracy of ~0.50, assuming a moderate heritability of 0.21 (Richardson *et al.* 2021b).

#### Discussion

We have developed two GHG-based subindexes that can be used to select for reduced emissions. The advantage of our approach is that it utilises the EBVs of traits currently estimated through the national genetic evaluation service and therefore does not require a direct CH<sub>4</sub> trait. Although we apply the method to Holsteins in the present paper, it can be adapted to derive weights for other dairy breeds, such as Jerseys and crossbreds, although the method should be validated in these breeds before implementation. This approach enables emission mitigation strategies through



**Fig. 1.** GHG<sub>index</sub> subindex scores among the top 300 BPI bulls. The solid vertical line is the average BPI (333) in the top 30 bulls and the dotted vertical line in the average BPI (312) for the top 30 BPI bulls with low GHG index values.



**Fig. 2.** Relationship between GHG<sub>index</sub> and SF<sub>6</sub> methane phenotypes, corrected for days in milk, batch-year and parity, in 464 cows located at the Ellinbank SmartFarm (Melbourne, Vic., Australia).

genetics to be implemented immediately, with the option of including a CH<sub>4</sub> trait when available. Our results showed that there is little to no advantage in including the current residual CH<sub>4</sub> trait, although this is expected to change with a higher-reliability residual CH<sub>4</sub> trait. It is expected that a residual CH<sub>4</sub> trait may be available in the future (Manzanilla-Pech *et al.* 2021; Richardson *et al.* 2021b). However, this is still a significant challenge, as CH<sub>4</sub> phenotypes are expensive and laborious to measure and combining international datasets is challenging, with multiple measuring apparatus and techniques being used in data CH<sub>4</sub> collection internationally, typically on a single breed (University of Guelph 2016).

#### *Relationship between GHG indexes*

The high correlation between the two calculated subindexes, GHG<sub>index</sub> and GHG<sup>+</sup><sub>index</sub>, showed that the RMP trait has a small effect on the index. This was expected, as the RMP trait has a low accuracy and a standard deviation of 5.0, whereas, for example, the standard deviation of the survival component of the GHG subindex was 28.36. However, as the RMP EBVs become more accurate, it is expected that the effect of the RMP traits within the index will increase proportional to the standard deviation and that the two GHG subindexes will further differentiate. We assumed that because the GHG subindexes and residual CH<sub>4</sub> EBV are both measured in CO<sub>2</sub>-eq and that the index components can be added together to calculate GHG<sub>index</sub><sup>+</sup>. However, further analysis is required to optimise the residual CH<sub>4</sub> EBVs within GHG<sub>index</sub><sup>+</sup>. Conceptually, the inclusion of RMP traits in the subindex is important (Knapp *et al.* 2014). The GHG subindex index will result in more environmentally efficient animals; however, selection on a direct CH<sub>4</sub> trait will capture the variation in CH<sub>4</sub> that exists between animals, resulting in a further reduction in CH<sub>4</sub> emissions. Further improvements to the GHG index and an increased availability of CH<sub>4</sub> phenotypes may help increase the accuracy of the index, and thereby the proportion of between-animal genetic variance captured by the index that can be utilised for selection. Although the effect of the RMP impact is minimal, these results have shown that we can use genetic selection to begin reducing emissions immediately. The GHG<sub>index</sub> method offers an option to include a sustainability component in the national breeding objective, with minimal compromises to advances in BPI and profit. In dairy systems where it is not possible to obtain accurate CH<sub>4</sub> phenotypes, the GHG<sub>index</sub> offers a valuable alternative that can be implemented simply and immediately, as it utilises EBVs currently available through the national genetic services.

#### *Relationships between GHG indexes and current indexes*

The relationships between the GHG indexes (GHG<sub>index</sub> and GHG<sup>+</sup><sub>index</sub>) and the current national selection indexes (BPI and HWI) are favourable. The strong favourable correlations between HWI and GHG subindexes support the hypothesis that selection for improved efficiency and survival is a viable mitigation option, as previous studies have identified (Wall *et al.* 2010; Pryce and Haile-Mariam 2020). Although the HWI

and BPI are strongly correlated (0.93), some re-ranking does occur between the indexes. The HWI places greater selection emphasis on health and functional traits, such as 5% on feed saved and ~40% on fertility and survival. Comparatively, the relative index weight in BPI for feed saved, and fertility and survival are 2.5% and 30% respectively (DataGene). The difference in comparative weighting also explains the differences in correlation with the GHG subindexes. Both the GHG subindexes and HWI place additional weight on survival and efficiency traits. However, the GHG subindexes also penalise higher production (milk, fat and protein). Selection on the HWI would result in favourable gains in the GHG subindexes. For the BPI, the correlations with the GHG subindexes were negligible (−0.02). However, in the top 50 bulls, 10 ranked negative on the GHG subindexes and a considerable number of bulls within the top 300 rank negative or close to 0 (Fig. 1). When considering the HWI, the GHG subindexes have a favourable negative correlation (−0.36) meaning that the higher the HWI, the less GHGs are emitted. As expected, on the basis of the favourable correlation between HWI and GHG, all but one of the top HWI Holstein bulls (within the top 300 BPI bulls) had a negative GHG subindex value, with one bull that was positive having a value very close to zero. This presents an opportunity to select for bulls that are profitable and also low GHG emitters.

#### *Correlations between GHG indexes and index traits*

As expected, the GHG subindexes are strongly correlated with the traits used in the index development, as well as with traits independent of index development that are suspected to have mitigation properties. Fertility was not included in the GHG subindexes as the CH<sub>4</sub> coefficient previously calculated only accounted for a portion of the effects of fertility, with all effects related to a reduction in replacements being accounted for through the survival EBV (Richardson *et al.* 2021a). However, there is a strong favourable effect between the GHG subindexes and fertility. This large favourable effect is also seen with heat tolerance (Nguyen *et al.* 2017), a trait defined as a rate of decline in milk, fat and protein yields per unit increase in temperature–humidity index. Heat tolerance is not included in the BPI and has a strong negative correlation with production traits, similar to the GHG subindexes. This suggests that selection only for a lower GHG subindex would result in decreases in production. However, by combining the GHG subindex with HWI or BPI, we can ensure that both genetic gain in profitable traits and decreases in gross emissions are achieved. Historically, unfavourable correlations among traits have been overcome by simultaneous inclusion in selection indexes, such as with fat and protein yield, although genetic gain is seen at a slower rate (Miglior *et al.* 2005). Type and production traits are also unfavourably correlated with the GHG subindexes, whereas more functional traits such as calving ease and mastitis resistance are favourably correlated. This suggests that transitioning some selection emphasis from type and production traits is favourable for environmental impact, and as Miglior *et al.* (2017) suggested, the selection for

robust animals is vital for the future sustainability of the dairy industry.

#### *Validation*

The validation methods of this research were limited by the small dataset size, as only the GHG<sub>index</sub> subindex could be compared with the SF<sub>6</sub> CH<sub>4</sub> data. However, as the GHG<sub>index</sub> and GHG<sup>+</sup><sub>index</sub> subindexes were highly related, it is expected that a similar accuracy may be obtained for the GHG<sup>+</sup><sub>index</sub> subindex. With an increase in dataset size to where a cross-validation may be conducted without jeopardising the accuracy of the RMP EBV or validity of the analysis, a validation may be also conducted on the GHG<sup>+</sup><sub>index</sub> subindex with a higher confidence. The reliability of the GHG<sub>index</sub> is sufficient for including the subindex in selection strategies. However, the accuracy of the GHG<sub>index</sub> to select for direct CH<sub>4</sub> is low. This is primarily due to the small dataset, as obtaining accurate CH<sub>4</sub> phenotypes on a large number of individual animals is challenging. In Australia, CH<sub>4</sub> phenotypes are currently measured on a reference population of 464 females, which is considerably lower than the suggested 5000-cow female reference population required for a moderately heritable trait to reach the same level of reliability (0.25) as the GHG subindex (González-Recio *et al.* 2014).

#### *Practical decision-making*

The GHG subindex may be used in practice to allow farmers to make environmentally conscious breeding decisions. The developed GHG<sub>index</sub> subindex has many opportunities for implementation, with minimal compromise to profit (Fig. 1). Within the BPI, the GHG<sub>index</sub> subindex could be included as an additional subindex and, although HWI is already favourably correlated with the GHG subindexes, inclusion of the GHG<sub>index</sub> subindex in HWI would put additional weight on traits based on their environmental impact (i.e. favouring longevity and efficiency). Additionally, these weights may be used in culling decisions to differentiate between two animals of similar genetic merit, as the higher emitter may be removed from the herd or breeding stock. These GHG<sub>index</sub> subindex values may be used on a magnitude or range basis. For example, the GHG<sub>index</sub> subindex may be used to sort cows into high-, medium-, low- and negative-emitter ranges. While using only bulls or animals with negative GHG<sub>index</sub> subindex values may not be possible at this time as this would result in increased inbreeding, farmers could use this range ranking system as a way of selecting for lower-emitting cows, leading towards a more environmentally conscious industry.

#### *Future index weights*

The traits within the GHG based subindexes are weighted based on their environmental impact. This weighting scheme is an alternative to the traditional economic index, which weights traits on the basis of profit. Alternative weighting options have been suggested, including social impact and farmer preference (Nielsen and Amer 2007). This has been implemented in the current Australian Breeding Objective through the HWI and

previously through the (now discontinued) Type Weighted Index (Byrne *et al.* 2016). Using survey approaches described by Martin-Collado *et al.* (2015), weights were assigned to traits on the basis of the priority and preferences of farmers, which grouped farmers into three sections (profit based, health based, and type based). Multi-source weighting allows the weights within an index to be developed on the basis of a subset of conditions. Those conditions may be economic, social, environmental or preference based, with the final weight applied to a trait being an aggregate of these weights. This should be considered when including the GHG<sub>index</sub> subindex in the national selection indexes (BPI and HWI) and not just as a supplementary selection decision tool. The GHG subindexes offer an additional option for weighting traits, on the basis of environmental impact. In theory, the GHG indexes are effectively weighting the traits twice. First, on the basis of economic analysis, and second, on the basis of environmental impact. However, in practice we are adjusting the index weight so that it is no longer purely economic based, but instead an aggregate weight of environmental and economic factors. This method may be especially useful to develop breeding strategies for traits with low economic values in breeds where expensive and laborious phenotypes, such as CH<sub>4</sub>, are not currently collected. Ideally, further breed-specific validations should be conducted, but as the majority of data has been collected on Holsteins, validation in other breeds is not possible at this time. As interest to include traits with low direct economic value in national selection indexes grows, further investigation is required to determine optimal inclusion methods.

#### **Conclusions**

This research has proposed a GHG subindex using EBVs currently estimated through the national genetic service and previously derived index weights that describe the change in CO<sub>2</sub>-eq associated with a unit change in each trait. The final set of EBVs selected for inclusion in the GHG subindex were milk, fat and protein yield, survival and feed saved as these traits had an independent effect on emissions. A further modification was to include a direct CH<sub>4</sub> trait in the GHG subindex, which was a RMP EBV. The two GHG subindexes were highly correlated, suggesting the RMP had a minimal impact on the GHG index, which was expected due to the low accuracy of the RMP EBV. The GHG subindexes had high reliabilities; however, the accuracy of the subindexes to predict CH<sub>4</sub> was reasonably low. The correlations between the GHG subindexes and current national selection indexes (HWI and BPI) suggest that selection strategies using HWI are expected to reduce emissions, whereas selection on BPI results in negligible changes in either GHG subindex. Type and production traits were unfavourably correlated with the GHG based subindexes, whereas traits associated with longevity, health, fertility and efficiency were favourable correlated. The GHG subindex may be included in the national selection index or used independently in practice by farmers to make environmentally conscious breeding and culling decisions, with minimal compromise to profit. Therefore, as the GHG subindex does not necessarily

require a direct CH<sub>4</sub> trait to lower the environmental impact of dairy cattle, selection for more sustainable dairy cattle may be promptly implemented until sufficient data are collected on CH<sub>4</sub>.

### Conflicts of interest

The authors declare no conflicts of interest.

### Acknowledgements

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# **Chapter 5:**

## **Reducing GHG Emission Through Genetic Selection in the Australian Dairy Industry**

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## Reducing greenhouse gas emissions through genetic selection in the Australian dairy industry

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### ABSTRACT

This research explores possible options to reduce greenhouse gas (GHG) emissions in the Australian dairy industry by (1) including an environmental component in the national breeding program and (2) estimating the economic and environmental impacts of implementation of the subsequent indexes. A total of 12 possible selection indexes were considered. These indexes were developed to predict changes in gross per-animal methane production (using 3 scenarios depending on availability and efficacy of a direct methane trait breeding value prediction) with 4 different carbon prices, integrating them into an augmentation of the current conventional national selection index. Although some economic response is lost with inclusion of the GHG subindexes in the Balanced Performance Index, options do exist where this loss is marginal and, even in scenarios where all selection pressure is based on the environmental weighting, economic progress is still made in all cases. When including environmental traits within an index, if a relatively low percentage of economic gain or index progression is sacrificed, then approximately 40 to 50% of the maximum possible reductions in emissions may be achieved. This concurrent selection of estimated breeding values that have a correlated favorable response in emissions in addition to direct selection on a residual methane trait allows a high level of methane reduction to be achieved with a realized cost to farmers that is far lower than the economic value placed on carbon. By implementing a GHG subindex in the national breeding program, we can achieve up to a 7.9% decrease in residual methane and 9 times the reduction in gross emissions in 10 yr, compared with the current breeding program, with

little to no cost to farmers. By 2050, selection based on one of the more moderate index scenarios at a carbon price of AUD\$250/t (AUD\$1 = US\$0.71), or opportunity cost to farmers of AUD\$87.22, will reduce gross emissions by 8.23% and emissions intensity by 21.25%, therefore offering a mitigation strategy that will be effective at reducing emissions with little compromise to profit.

**Key words:** sustainability, methane emission, environment, economic impact, selection index

### INTRODUCTION

In 2016, Australia committed to reduce greenhouse gas (GHG) emissions by 26 to 28% of 2005 levels by the year 2030 (Paris Agreement, 2016). This target relies largely on a decrease in the 3 major GHG, carbon dioxide (CO<sub>2</sub>), methane, and nitrous oxide, which account for 81%, 10%, and 7% of the global GHG inventory, respectively (<https://www.epa.gov/ghgemissions/overview-greenhouse-gases>).

Enteric methane produced as a by-product of feed fermentation (Appuhamy et al., 2016) is the primary GHG targeted for reduction, as it accounts for 57% of the Australian dairy industry's emissions (UNFCCC, 2018). Genetic selection for lower enteric methane production may provide a permanent and cumulative solution to reduce GHG emissions, and several strategies could be incorporated into breeding programs to target a reduction in emissions (Wall et al., 2010; Basarab et al., 2013; Pryce and Haile-Mariam, 2020).

National selection indexes are a powerful tool for modifying the trajectory and rate of genetic change across multiple traits. These indexes are typically constructed with a focus on how traits are expected to influence the direct profitability or efficiency of production. For genetic selection to contribute to environmental gains, these national indexes will need to be expanded and modified to consider how existing and novel selection criteria can be optimally weighted to

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efficiently allocate selection efforts among traits that affect only profit, traits that affect only environmental outcomes, and traits that affect both (Boichard and Brochard 2012; Martin-Collado et al., 2015).

Three of the challenges in accommodating environmental traits are as follows: (1) identifying appropriate selection criteria that are available on a large number of selection candidates; (2) deriving meaningful weights for these selection criteria; and the focus of the current paper; and (3) evaluating the consequences of multi-trait selection directly on the traits in the index and on other correlated traits.

Previous studies have investigated possible methane trait definitions (Breider et al., 2019; González-Reico et al., 2020; Manzanilla-Pech et al., 2021), and some of these may be suitable for inclusion in the Australian national dairy breeding objective (Richardson et al., 2021b). Richardson et al. (2021b) defined candidate enteric methane traits to reduce environmental impact without severely affecting other valuable traits such as production, health, and fertility within the context of the Australian national dairy breeding program. Under these criteria, a residual methane trait phenotypically corrected for energy-corrected milk was proposed due to its heritability ( $0.21 \pm 0.10$ ) and the potential ease of incorporating the trait within future indexes, as it is unexpected to have substantial correlations with other economically relevant traits. Emissions may also be reduced indirectly through correlated traits known to have a favorable effect on reducing enteric methane. Survival and feed saved (kg of DM/yr; Pryce et al., 2015) are traits currently included in the national selection indexes that have been shown to reduce emissions due to their independent effects of reducing methane through fewer replacements and lower feed requirements, respectively (Richardson et al., 2021a).

Two selection indexes are commonly used by Australian dairy farmers: the Balanced Performance Index (BPI) and the Health Weighted Index (HWI). The BPI is an economic-based index aimed at improving the overall profit on farm, whereas the HWI places additional emphasis on fertility, health, and feed efficiency. Byrne et al. (2016) describe the index development, which was recently updated with current industry parameters and economic inputs (Axford et al., 2021). The HWI was developed by incorporating the 1000Minds approach (1000Minds Ltd., Dunedin, New Zealand), which also gives traits a noneconomic value-based farmer preference for improvements, reflecting the intrinsic interests of farmers separate from economics and profit (Martin-Collado et al., 2015). This willingness to sacrifice potential economic gains

to achieve progress in traits related to management or social purposes is useful for deriving alternative index weights based not exclusively on economic benefits, such as for environmental traits. Although there is currently no predetermined economic value for carbon within Australia, social pressures to responsibly reduce emissions place a new pressure on the industry to adapt their farming practices, including modification of their genetic selection choices.

The effects of including an environmental component in the national selection index needs to be estimated before a strategy to reduce GHG through genetic selection can be efficiently implemented. The aim of this research was to explore possible options to reduce GHG emissions in the Australian dairy industry, using adapted versions of the BPI that include GHG subindexes developed using a combination of currently evaluated traits in addition to a direct residual methane trait, while maintaining a profitable dairy animal through (1) including an environmental component in the national breeding program and (2) estimating the economic and environmental impacts of implementation of the subsequent indexes.

## MATERIALS AND METHODS

In Australia, the current primary national selection index is the BPI (Axford et al., 2021); therefore, the implementation of possible GHG subindexes within the BPI were examined and compared with the most recent BPI and HWI indexes, noting that HWI has more of a desired-gains approach that is focused on improving health and fertility traits. A total of 12 possible selection indexes were considered. These indexes were developed by including 3 variations of a GHG sub-index: (1) considering current selection criteria alone, referred to as the base GHG subindex ( $\text{GHG}_{\text{index}}$ ); (2) current criteria plus a novel but low-accuracy direct residual methane trait ( $\text{GHG}_{\text{index}}^+$ ); (3) current criteria plus a future higher-accuracy residual methane trait ( $\text{GHG}_{\text{index}}^s$ ). For all versions, the subindexes were weighted according to their effect on gross methane per cow per lactation.

The 3 options were then evaluated when integrated into the current BPI index, with 4 different carbon prices used to maximize genetic progress in a holistic economic-based breeding goal. The environmental and economic impacts of the 12 index scenarios were estimated and compared with the 2 current national indexes. Additionally, selection solely on the GHG subindexes was considered, to determine the maximum magnitude of enteric residual methane reduction that could be achieved.

### GHG Subindex Development

A base GHG subindex, or  $\text{GHG}_{\text{index}}$ , was previously developed as described by Richardson et al. (2021a). Briefly, gross emissions coefficients ( $\mathbf{GV}$ ; Table 1) were estimated that describe the change in enteric methane attributed to traits currently under selection in Australian dairy cattle (expressed in  $\text{CO}_2$  equivalents,  $\text{CO}_2\text{-eq}$ ). Because these GV coefficients were estimated to be independent, they can be used as weights within an index to place noneconomic emphasis on traits with environmental impact. The calculated GV coefficients were applied to existing EBV shown to have an independent effect on enteric methane emissions and used to develop  $\text{GHG}_{\text{index}}$ , which has units of  $\text{CO}_2\text{-eq}$  emitted per cow per year:

$$\text{GHG}_{\text{index}} = \sum_{n=1}^i \text{GV}_i \times \text{EBV}_i, \quad [2]$$

where  $\text{GV}_i$  is the gross emissions coefficient (kg of  $\text{CO}_2\text{-eq/yr}$ ) for trait  $i$  (milk, fat, protein, survival, and feed saved) and  $\text{EBV}_i$  is the EBV for trait  $i$  (milk, fat, protein, survival, and feed saved).

A preliminary residual methane trait was added to the index to determine the effect of including a direct methane trait on reducing emissions (i.e.,  $\text{GHG}_{\text{index}}^+$ ). The residual methane trait (kg of methane/yr) was previously developed by Richardson et al. (2021b) and defined as methane production phenotypically corrected for ECM. Thus,  $\text{GHG}_{\text{index}}^+$  is as follows:

$$\text{GHG}_{\text{index}}^+ = \text{GHG}_{\text{index}} + \text{GV}_{\text{RMP}} \times \text{EBV}_{\text{RMP}}, \quad [3]$$

where  $\text{GV}_{\text{RMP}}$  is the gross methane coefficient for residual methane, and  $\text{EBV}_{\text{RMP}}$  is the EBV for residual

methane phenotypically corrected for ECM (kg of methane/yr).

### Residual Methane Genomic EBV

The genomic prediction equation to calculate  $\text{EBV}_{\text{RMP}}$  was estimated using a reference population of 464 Holstein cows from Ellinbank, Australia (Richardson et al., 2021b). Measurements for methane production were performed using the  $\text{SF}_6$  tracer method previously described by Deighton et al. (2014), which was then corrected phenotypically for ECM to produce the residual methane phenotypes (Richardson et al., 2021b) and calculated as follows:

$$\text{RMP}_{\text{pECM}} = \text{MeP} - (\text{b}_{\text{pECM}} \times \text{ECM}), \quad [4]$$

where  $\text{RMP}_{\text{pECM}}$  is RMP phenotypically corrected for ECM, ECM is energy-corrected milk (kg/d),  $\text{b}_{\text{pECM}}$  is the linear regression coefficient of MeP on ECM; and MeP is methane production. The effects of 41,276 SNP on the Illumina Bovine 50K panel (Illumina Inc.) were estimated as  $\hat{\beta} = \mu + \mathbf{Z}'(\mathbf{Z}\mathbf{Z}')^{-1}\hat{\mathbf{g}}$ , where  $\mathbf{Z}$  is the genotype matrix (464 individuals  $\times$  41,276 SNP), and  $\hat{\mathbf{g}}$  is a vector of descaled direct genomic value estimated using GBLUP (VanRaden, 2008). The vector  $\hat{\mathbf{g}}$  was applied to the genotypes of 3,412 Holstein bulls to calculate  $\text{EBV}_{\text{RMP}}$ . It was assumed that  $\text{EBV}_{\text{RMP}}$  had an accuracy of 0.1, as estimated in the independent cross-validation described by Richardson et al. (2021b).

All other EBV for the 3,412 registered Holstein bulls used in this study were provided by DataGene Ltd. (Bundoora, Victoria, Australia). Bulls were born between 2010 and 2015. The EBV used in this analysis included milk volume, milk fat, milk protein, survival, fertility, feed saved, mastitis resistance, somatic cell

**Table 1.** Gross emissions coefficients (kg of  $\text{CO}_2$  equivalents,  $\text{CO}_2\text{-eq}$ ) and subsequent relative emphasis for traits shown to have an independent effect of greenhouse gas (GHG) emissions in dairy cattle

Trait	Gross emissions coefficient, <sup>1</sup> kg of $\text{CO}_2\text{-eq}$			Relative emphasis, %		
	$\text{GHG}_{\text{index}}$	$\text{GHG}_{\text{index}}^+$	$\text{GHG}_{\text{index}}^{\text{S}}$	$\text{GHG}_{\text{index}}$	$\text{GHG}_{\text{index}}^+$	$\text{GHG}_{\text{index}}^{\text{S}}$
Protein	1.97	1.97	1.97	9.44	7.31	3.95
Fat	3.19	3.19	3.19	32.33	18.45	10.60
Milk	0.04	0.04	0.04	8.34	6.93	3.74
Survival	-10.19	-10.19	-10.19	26.63	20.26	9.99
Feed saved	-0.53	-0.53	-0.53	23.26	20.66	14.75
RMP <sup>2</sup>	0.00	28.00	0.00	0.00	26.38	0.00
RMPS <sup>3</sup>	0.00	0.00	28.00	0.00	0.00	56.95

<sup>1</sup>Gross emissions coefficients were previously calculated by Richardson et al. (2021a).  $\text{GHG}_{\text{index}}$  was developed as described by Richardson et al. (2021c) and updated to include a gross emissions value for residual methane ( $\text{GHG}_{\text{index}}^+$ ) as well as a simulated residual methane trait with higher accuracy ( $\text{GHG}_{\text{index}}^{\text{S}}$ ).

<sup>2</sup>RMP is the residual methane production EBV as defined by Richardson et al. (2021b).

<sup>3</sup>RMPS is the simulated residual methane production EBV with higher accuracy.

count, milking speed, milking temperament, overall type, mammary system, pin set, and udder depth, and were extracted from the April 2020 national genetic evaluation. Details of the traits evaluated can be found on the DataGene website ([www.datagene.com.au](http://www.datagene.com.au)).

### Converting Residual Methane EBV to BPI Units

Residual methane EBV were estimated in units of grams per day, taken as the recorded average daily methane emitted per cow after phenotypic adjustment for ECM. These EBV were rescaled to the units of the BPI and the GHG subindex. Because the BPI and HWI are annual measurements (i.e., the aggregate is a measure of profit per year), it was necessary to convert residual methane EBV from grams of methane per day to kilograms of methane per year. A conversion constant ( $c$ ) was defined as the number of full equivalent days of methane production (at the time of research data collection) per year and was calculated as

$$c = t \times \sum \left( \frac{DMI_i}{DMI_R} \right), \quad [5]$$

where  $t$  is the time period to which the EBV is converted,  $DMI_i$  is dry matter intake at  $i$  days in milk, and  $DMI_R$  is the DMI at the average days in milk when methane phenotypes were collected ( $DIM = 113$ ).

### Calculating Emissions Coefficient for Residual Methane

The GV for residual methane was calculated as 1 kg of additional methane produced per 1-unit increase in residual methane, multiplied by the global warming potential of methane to carbon (1 kg of methane  $\times$  28 CO<sub>2</sub>-eq/1 kg of methane; Gerber et al., 2013).

### Simulating Residual Methane EBV

One of the challenges of implementing a residual methane EBV in an index is the small number of phenotypes that are available on genotyped individuals, resulting in low accuracy of the performed genomic

evaluation (Goddard et al., 2011). In the future, it is anticipated that additional phenotypes for methane will become available through various strategies, such as expanding data sets, or more extensive use of predictors.

To quantify the effect of future higher genomic prediction accuracies, an additional GHG subindex (i.e.,  $GHG_{index}^S$ ) was investigated to determine the influence of including a more accurate residual methane trait into the GHG subindex and subsequently the BPI. Residual methane EBV were simulated, based on the expected accuracy of a residual methane trait with a heritability of 0.21 (Richardson et al., 2021b) and reference population sizes of 1,000, 3,000, 5,000, 10,000, 50,000, and 100,000 animals, using methods described by Goddard et al. (2011).

Using the formula proposed by Goddard et al. (2011), it was assumed that the predicted accuracy can be estimated as  $r = \sqrt{\frac{\theta}{1 + \theta - r^2 h^2}}$  with  $\theta = \frac{Th^2}{M_e}$ , where  $T$  is the size of the reference population,  $h^2$  the trait heritability, and  $M_e$  the effective number of independent chromosome segments (Table 2).  $M_e$  can be estimated using the effective population size and chromosome segment length. We assumed an effective population size of 100 and a chromosome length of 30 (Zimin et al., 2009). Residual methane EBV were simulated using the `rnorm_pre()` function in R (R Core Team, 2021) by multiplying the predicted accuracy for a reference population size of 3,000 animals with the standard deviation of the previously estimated residual methane EBV and assuming a correlation of 0.4 between residual methane and liveweight (Breider et al., 2019). The simulated residual methane trait was then added to the base GHG index using the estimated GV for residual methane to develop the simulated GHG subindex,  $GHG_{index}^S$ , and defined as

$$GHG_{index}^S = GHG_{index} + GV_{RMP} \times EBV_{S,RMP}, \quad [6]$$

where  $GV_{RMP}$  is the gross methane coefficient for residual methane, and  $EBV_{S,RMP}$  is the simulated residual methane EBV with a higher accuracy through the increased reference population size (3,000 animals).

**Table 2.** Parameters used to estimate prediction accuracy, using methods described by Goddard et al. (2011)

Constant	Value	Reference
Heritability, residual methane	0.21	Richardson et al., 2021b
Chromosome segment length (LN. Chr)	30	Zimin et al., 2009
Effective population size (Ne)	100	Zimin et al., 2009
Effective number of independent chromosome segments (Me)	1,132.43	
Prediction accuracy of cross-validation	0.1	Richardson et al., 2021b

**Table 3.** Weight and relative emphasis of the greenhouse gas (GHG) index when included within the Balanced Performance Index (BPI) at carbon price of AUD\$150/t, AUD\$250/t, AUD\$500/t, and AUD\$1,000/t

Scenario <sup>1</sup>	Index weight				Relative emphasis			
	\$150/t	\$250/t	\$500/t	\$1,000/t	\$150/t	\$250/t	\$500/t	\$1,000/t
BPI.1	-0.15	-0.25	-0.50	-1.00	3.77	5.25	10.56	20.01
BPI.2	-0.15	-0.25	-0.50	-1.00	4.41	6.39	13.39	23.56
BPI.3	-0.15	-0.25	-0.50	-1.00	8.20	11.43	23.67	41.40

<sup>1</sup>BPI.1, BPI.2 and BPI.3 describe scenarios where  $GHG_{index}$ ,  $GHG^+_{index}$ , and  $GHG^S_{index}$  have been included in the BPI, respectively.  $GHG_{index}$  was developed as described by Richardson et al. (2021c) and updated to include a gross emissions value for residual methane ( $GHG^+_{index}$ ) as well as a simulated residual methane trait with higher accuracy ( $GHG^S_{index}$ ).

### Inclusion in National Selection Index

The 3 GHG subindexes were included in the BPI with 4 different carbon prices driving the economic weighting applied to methane. The 3 scenarios represent the scenarios when no or insufficient records for residual methane are available (**BPI.1**), using the data currently available for residual methane (**BPI.2**), and a scenario with an increased accuracy of residual methane EBV (**BPI.3**). The investigated index scenarios were as follows:

$$BPI.1 = BPI + (EV_C \times GHG_{index}); \quad [7]$$

$$BPI.2 = BPI + (EV_C \times GHG^+_{index}); \quad [8]$$

$$BPI.3 = BPI + (EV_C \times GHG^S_{index}), \quad [9]$$

where the component indexes are as previously described, and  $EV_C$  is the carbon price at AUD\$150, AUD\$250, AUD\$500, and AUD\$1,000/t (AUD\$1 = US\$0.71). Considerable variation currently exists in the price of carbon and future predictions of that price; for example, AUD\$55/t has been used as the price in Canada (Government of Canada, 2021), whereas studies of the true long-term cost of carbon have produced much higher values, up to AUD\$550 depending on the country, year, and sector of implementation (Stern and Stiglitz, 2021; World Bank, 2021). The effect of using the GHG subindexes as independent selection tools separate from the BPI was also explored.

### GHG Subindex Included in National Indexes

The weights applied to traits included within BPI.1, BPI.2, and BPI.3 are presented in 2 ways. First, as the relative weight that the GHG subindex receives within the BPI (Table 3), and, second, as the total weight that each trait will receive when a GHG subindex is included in the BPI by combining both the economic and environmental weights (Table 4). For example, the final weight of protein within BPI.1 is equal to

the economic value of protein (AUD\$6.76), which is the current weight protein receives in BPI, plus additional emphasis provided by converting the GV into an economic value using the respective carbon price. For example, in the scenario where the carbon price is AUD\$150/t, it was calculated as  $1.97 \text{ kg CO}_2\text{-eq/kg protein} \times -0.15/\text{kg CO}_2\text{-eq}$ .

### Relative Emphasis

The relative emphasis of each trait and subindexes for every variant of the BPI was calculated using the approach of Zhang et al. (2021), which accounts for the accuracy of the EBV as well as the (favorable or antagonistic) relationships between traits in contrast to traditional approaches that are often a simple multiplication of the relative contribution of each trait's economic value (converted to absolute value) by its genetic standard deviation (SD). Here, we applied the method of Zhang et al. (2021) using correlations between the EBV. The resulting trait emphasis values more accurately present the true selection pressure each trait receives within the given index.

### Environmental and Economic Response to Selection

Current and future rates of genetic progress in novel GHG traits and subindexes were modeled using EBV for Australian dairy cattle. A regression analysis was used to compare selection on the BPI options that integrate GHG subindexes with the response expected from selection on BPI alone. Scott et al. (2021) reported that the annual rate of genetic gain in BPI since 2013 ranged between 0.11 and 0.22 genetic SD per year for Holstein cows and bulls, respectively. Consequently, we assumed that a 1-SD improvement (AUD\$84.06; Axford et al., 2021) in BPI would be achieved over around 10 yr of selection. The responses in BPI units achieved by selection on each of the considered indexes, as well as the total CO<sub>2</sub>-eq reduction achieved by selection for each index, are presented.

**Table 4.** Economic weight applied to each trait when the relative weight for each trait within the greenhouse gas (GHG) index was applied directly to traits currently included in the national indexes at AUD\$150/t of carbon (C), AUD\$250/t of C, AUD\$500/t of C, and AUD\$1,000/t of C; the final economic weight was calculated by combining the economic value of the trait with the gross emissions coefficient by converting the gross emissions coefficients to economic units by multiplying by the carbon price

Trait	National <sup>1</sup>			EV <sub>C</sub> <sup>2</sup> = \$150/t			EV <sub>C</sub> = \$250/t			EV <sub>C</sub> = \$500/t			EV <sub>C</sub> = \$1,000/t				
	BPI	HWI	BPI.1 <sup>3</sup>	BPI.2	BPI.3	BPI.1	BPI.2	BPI.3	BPI.1	BPI.2	BPI.3	BPI.1	BPI.2	BPI.3	BPI.1	BPI.2	BPI.3
Protein	6.76	4.36	6.46	6.46	6.46	6.27	6.27	6.27	5.78	5.78	5.78	4.79	4.79	4.79	4.79	4.79	4.79
Fat	2.08	1.35	1.60	1.60	1.60	1.28	1.28	1.28	0.49	0.49	0.49	-1.11	-1.11	-1.11	-1.11	-1.11	-1.11
Milk	-0.11	-0.07	-0.116	-0.12	-0.12	-0.12	-0.12	-0.12	-0.13	-0.13	-0.13	-0.15	-0.15	-0.15	-0.15	-0.15	-0.15
Survival	7.2	7.2	8.73	8.73	8.73	9.75	9.75	9.75	12.30	12.30	12.30	17.39	17.39	17.39	17.39	17.39	17.39
Fertility	6.94	14.11	6.94	6.94	6.94	6.94	6.94	6.94	6.94	6.94	6.94	6.94	6.94	6.94	6.94	6.94	6.94
SCC	0.69	0.69	0.69	0.69	0.69	0.69	0.69	0.69	0.69	0.69	0.69	0.69	0.69	0.69	0.69	0.69	0.69
Mastitis resistance	6.75	6.75	6.75	6.75	6.75	6.75	6.75	6.75	6.75	6.75	6.75	6.75	6.75	6.75	6.75	6.75	6.75
Milking speed	5.02	5.02	5.02	5.02	5.02	5.02	5.02	5.02	5.02	5.02	5.02	5.02	5.02	5.02	5.02	5.02	5.02
Milking temperament	3.6	3.6	3.6	3.6	3.6	3.6	3.6	3.6	3.6	3.6	3.6	3.6	3.6	3.6	3.6	3.6	3.6
Mammary system	2.76	3.59	2.76	2.76	2.76	2.76	2.76	2.76	2.76	2.76	2.76	2.76	2.76	2.76	2.76	2.76	2.76
Udder depth	0.82	0.00?	0.82	0.82	0.82	0.82	0.82	0.82	0.82	0.82	0.82	0.82	0.82	0.82	0.82	0.82	0.82
Overall type	1.36	1.36	1.36	1.36	1.36	1.36	1.36	1.36	1.36	1.36	1.36	1.36	1.36	1.36	1.36	1.36	1.36
Pin set	0.78	0.78	0.78	0.78	0.78	0.78	0.78	0.78	0.78	0.78	0.78	0.78	0.78	0.78	0.78	0.78	0.78
Feed saved	0.19	0.38	0.27	0.27	0.27	0.32	0.32	0.32	0.45	0.45	0.45	0.72	0.72	0.72	0.72	0.72	0.72
RMP <sup>4</sup>	0.00	0.00	0.00	-4.20	0.00	0.00	-7.00	0.00	0.00	-14.00	0.00	0.00	-28.00	0.00	-28.00	0.00	-28.00
RMPS <sup>5</sup>	0.00	0.00	0.00	0.00	-4.20	0.00	0.00	-7.00	0.00	0.00	-14.00	0.00	0.00	-28.00	0.00	-28.00	-28.00

<sup>1</sup>Economic weights calculated through NBO Review DataGene Ltd. (Bundoora, Victoria, Australia), 2020.

<sup>2</sup>EV<sub>C</sub> is the carbon price.

<sup>3</sup>BPI.1, BPI.2, and BPI.3 describe scenarios where GHG<sub>index</sub>, GHG<sup>+</sup><sub>index</sub>, and GHG<sup>S</sup><sub>index</sub> have been included in the Balanced Performance Index, respectively. The GHG<sub>index</sub> was developed as described by Richardson et al. (2021c) and updated to include a gross emissions value for residual methane (GHG<sup>+</sup><sub>index</sub>) as well as a simulated residual methane trait with higher accuracy (GHG<sup>S</sup><sub>index</sub>).

<sup>4</sup>RMP is the residual methane production EBV as defined by Richardson et al. (2021b).

<sup>5</sup>RMPS is the simulated residual methane production EBV with higher accuracy.

The opportunity costs of carbon emissions reductions, or costs to farmers, achieved through breeding for each index with emphasis on emissions were derived by taking the ratio of the reduction in BPI units achieved in AUD\$ per cow per year relative to the emissions reduction in CO<sub>2</sub>-eq per cow per year when compared with the same index without any emphasis on emissions, as follows:

$$Opportunity\ Cost_i = \frac{\Delta Index\ Gain(\$)}{\Delta Emissions\ Reduction} \times 1,000\text{ kg/t}, \quad [10]$$

where  $\Delta Index\ Gain$  is the difference in the economic response to selection (AUD\$/SD BPI) between the primary index (BPI) and the  $i$ th selection index and  $\Delta Emissions\ Reduction$  is the difference in emissions response to selection (kg of CO<sub>2</sub>-eq/SD BPI) between the primary index (BPI) and the proposed  $i$ th selection indexes. The selection indices evaluated as  $i$  were HWI, GHG<sub>index</sub>, GHG<sup>+</sup><sub>index</sub>, GHG<sup>S</sup><sub>index</sub>, as well as BPI.1, BPI.2, and BPI.3 at carbon prices of AUD\$150/t, AUD\$250/t, AUD\$500/t, and AUD\$1,000/t.

## RESULTS

### Relative Percent Emphasis of GHG Subindexes

By constructing subindexes that include traits weighted based on their independent effects on emissions, we calculated the relative emphasis of each trait within the GHG subindexes (Table 1). Here we tested (1) milk, fat, protein, survival, and feed saved EBV as GHG<sub>index</sub>, (2) added an estimated residual methane trait as GHG<sup>+</sup><sub>index</sub>, and (3) included a simulated residual methane trait as GHG<sup>S</sup><sub>index</sub>, resulting in the following:

- (1) In GHG<sub>index</sub>, fat yield had the largest relative emphasis at 32%, with survival and feed saved contributing 27% and 23%, respectively. Protein (10%) and milk (8%) were more moderately weighted within the GHG<sub>index</sub>.
- (2) When an estimated residual methane trait was added to the GHG<sup>+</sup><sub>index</sub>, the relative weights shifted to residual methane having the largest emphasis (26%), with survival (20%), feed saved (21%), and fat (19%) having the next largest relative emphasis within the subindex. Milk and protein remained moderately weighted at 7%.
- (3) The inclusion of a simulated residual methane trait with a higher accuracy further increased the relative emphasis of residual methane to

57% within GHG<sup>S</sup><sub>index</sub>. Feed saved had the next largest emphasis at 15%, with survival and fat having a moderate emphasis of 10%. Milk and protein had small emphasis at 4%.

### Relative Emphasis of GHG Subindexes and Component Traits Within BPI

The next step was to augment the BPI to include the 3 subindexes described in the previous section; the economic weights in each of GHG subindex included in the BPI at varying carbon prices are presented in Table 3. At an economic value of AUD\$150/t of carbon the GHG<sub>index</sub>, GHG<sup>+</sup><sub>index</sub>, and GHG<sup>S</sup><sub>index</sub> received 3.77%, 4.40%, and 8.2% of the total selection emphasis within BPI, respectively. These relative weights increased proportional to the economic values placed on carbon of AUD\$500/t and AUD\$1,000/t in the GHG<sub>index</sub> (10.57% and 20.01%, respectively), GHG<sup>+</sup><sub>index</sub> (13.39% and 23.56%), and GHG<sup>S</sup><sub>index</sub> (23.67% and 41.40%).

The GHG subindexes are composed of EBV routinely evaluated in Australia, as well as a residual methane trait. The economic weights and subsequent relative emphasis of each trait within the BPI as a result of including the GHG subindexes are presented in Tables 4 and 5, respectively. Briefly, as the price of carbon increased, additional emphasis shifted onto fertility, survival, mastitis resistance (and SCC), and feed saved, whereas relative emphasis was lowered for production and type traits.

### Predicted Accuracy

The predicted accuracies of a residual methane trait with various reference population sizes are presented in Figure 1. The predicted accuracy of the residual methane trait ranged from 0.36 ( $n = 1,000$ ) to 0.88 ( $n = 100,000$ ). For a reference population of 3,000 animals, which was used to simulate the residual methane trait used as part of GHG<sup>S</sup><sub>index</sub>, the predicted accuracy was 0.54.

### Economic Response

The economic responses to selection on each index for a 1-SD improvement in BPI, or approximately 10 yr of selection, equivalent to AUD\$84.06 BPI units (\$AUD per cow per year), are presented in Figure 2. When the equivalent selection pressure of 1 SD of BPI was applied to HWI, the economic response (in BPI units) was AUD\$76.57. Minimal change in economic response was observed when the GHG<sub>index</sub> (AUD\$83.34), GHG<sup>+</sup><sub>index</sub> (AUD\$83.08), and GHG<sup>S</sup><sub>index</sub>

**Table 5.** Relative emphasis of traits included in the national selection indexes and when the greenhouse gas (GHG) subindexes were included in the Balanced Performance Index (BPI) at carbon prices AUD\$150/t, AUD\$250/t, AUD\$500/t, and AUD\$1,000/t

Trait	National			EV <sub>C</sub> <sup>1</sup> = \$150/t			EV <sub>C</sub> = \$250/t			EV <sub>C</sub> = \$500/t			EV <sub>C</sub> = \$1,000/t				
	BPI	HWI	BPI.1 <sup>2</sup>	BPI.2	BPI.3	BPI.1	BPI.2	BPI.3	BPI.1	BPI.2	BPI.3	BPI.1	BPI.2	BPI.3	BPI.1	BPI.2	BPI.3
Protein	19.94	10.55	16.96	15.68	12.99	15.95	14.87	11.47	13.48	12.59	8.52	9.53	8.63	4.61			
Fat	9.25	4.92	5.24	5.64	4.95	4.23	4.16	3.61	1.72	1.63	1.10	3.56	3.34	1.64			
Milk	14.11	7.37	14.21	13.14	10.88	14.25	13.29	10.25	14.16	13.23	8.95	13.93	12.62	6.74			
Survival	8.66	9.09	12.51	11.93	13.04	13.74	13.08	13.60	16.30	15.10	14.36	19.67	17.06	14.75			
Fertility	13.66	29.14	17.30	16.50	18.04	17.01	16.19	16.84	16.00	14.82	14.09	13.65	11.84	10.24			
SCC	4.86	5.10	6.62	6.32	6.91	6.52	6.20	6.45	6.13	5.08	5.40	5.23	4.53	3.92			
Mastitis resistance	7.33	7.69	8.77	8.37	9.15	8.63	8.21	8.54	8.12	7.52	7.15	6.93	6.01	5.19			
Milking speed	3.98	4.17	2.98	3.21	2.82	3.01	2.96	2.57	3.23	3.07	2.07	2.93	2.74	1.35			
Milking temperament	2.28	1.87	1.78	1.92	1.68	1.79	1.76	1.53	1.92	1.83	1.24	1.75	1.64	0.81			
Mammary system	5.28	7.24	3.43	3.91	3.26	3.45	3.83	3.32	3.71	3.52	3.19	3.36	2.91	2.56			
Udder depth	1.35	0.00?	1.46	1.39	1.52	1.43	1.36	1.42	1.35	1.25	1.19	1.15	1.00	0.86			
Overall type	2.43	2.57	1.67	1.90	1.59	1.68	1.86	1.61	1.80	1.71	1.55	1.64	1.41	1.25			
Pin set	1.65	1.74	1.07	1.15	1.01	1.08	1.06	0.92	1.16	1.10	0.74	1.05	0.98	0.48			
Feed saved	5.21	8.54	6.00	6.85	5.71	7.22	8.01	6.94	10.92	10.36	9.41	15.63	13.52	11.90			
RMP <sup>3</sup>	0.00	0.00	0.00	2.07	0.00	0.00	3.17	0.00	0.00	6.59	0.00	0.00	11.78	0.00			
RMPS <sup>4</sup>	0.00	0.00	0.00	0.00	6.44	0.00	0.00	10.92	0.00	0.00	21.03	0.00	0.00	33.70			

<sup>1</sup>EV<sub>C</sub> is the carbon price.

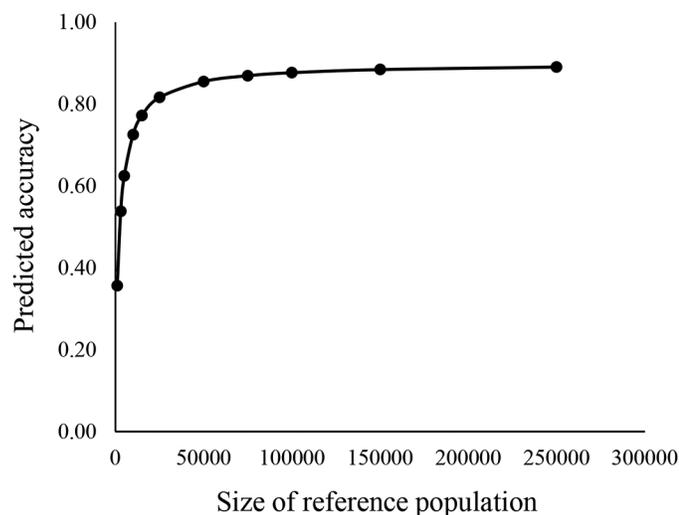
<sup>2</sup>BPI.1, BPI.2, and BPI.3 describe scenarios where GHG<sub>index</sub>, GHG<sup>+</sup><sub>index</sub>, and GHG<sup>S</sup><sub>index</sub> have been included in the BPI, respectively. The GHG<sub>index</sub> was developed as described by Richardson et al. (2021c) and updated to include a gross emissions value for residual methane (GHG<sup>+</sup><sub>index</sub>) as well as a simulated residual methane trait with higher accuracy (GHG<sup>S</sup><sub>index</sub>).

<sup>3</sup>RMP is the residual methane production EBV as defined by Richardson et al. (2021b).

<sup>4</sup>RMPS is the simulated residual methane production EBV with higher accuracy.

(AUD\$80.37) were included in the BPI at a carbon price of AUD\$150/t of carbon, and when  $\text{GHG}_{\text{index}}$  and  $\text{GHG}_{\text{index}}^+$  were included in BPI at a carbon price of AUD\$250/t (AUD\$82.18 and AUD\$81.50, respectively). An economic response similar to the HWI was observed when  $\text{GHG}_{\text{index}}^{\text{S}}$  (AUD\$75.53) was included in the BPI at a carbon price of AUD \$250/t and when  $\text{GHG}_{\text{index}}$  and  $\text{GHG}_{\text{index}}^+$  were included at a carbon price of AUD\$500/t (AUD\$77.84 and AUD\$75.72). A lower economic response was observed when  $\text{GHG}_{\text{index}}^{\text{S}}$  (AUD\$63.03) was included in the BPI at a carbon price of AUD\$500/t and for the  $\text{GHG}_{\text{index}}$  and  $\text{GHG}_{\text{index}}^+$  at a carbon price of AUD\$1,000/t (AUD\$67.62 and AUD\$62.96).

The lowest economic response (AUD\$47.11) was observed when  $\text{GHG}_{\text{index}}^{\text{S}}$  was included in the BPI at a carbon price of AUD\$1,000/t. Interestingly, economic responses of AUD\$20.73, AUD\$15.37, and AUD\$18.08 were estimated for selection solely on the  $\text{GHG}_{\text{index}}$ ,  $\text{GHG}_{\text{index}}^+$ , and  $\text{GHG}_{\text{index}}^{\text{S}}$ , respectively. For these indexes, significant genetic progress is achieved in fertility and survival, but at the expense of genetic progress in milk production traits (Table 5). Although economic response is lost with inclusion of the GHG subindexes in the BPI, options do exist where this loss is marginal, and, even in scenarios where all selection pressure is based on the environmental weighting, economic progress is still made in all cases.



**Figure 1.** Prediction accuracy of a simulated residual methane trait phenotypically corrected for ECM. Accuracy was predicted using methods described by Goddard et al. (2011), assuming a heritability of 0.21, chromosome segment length of 30, and effective population size of 100.

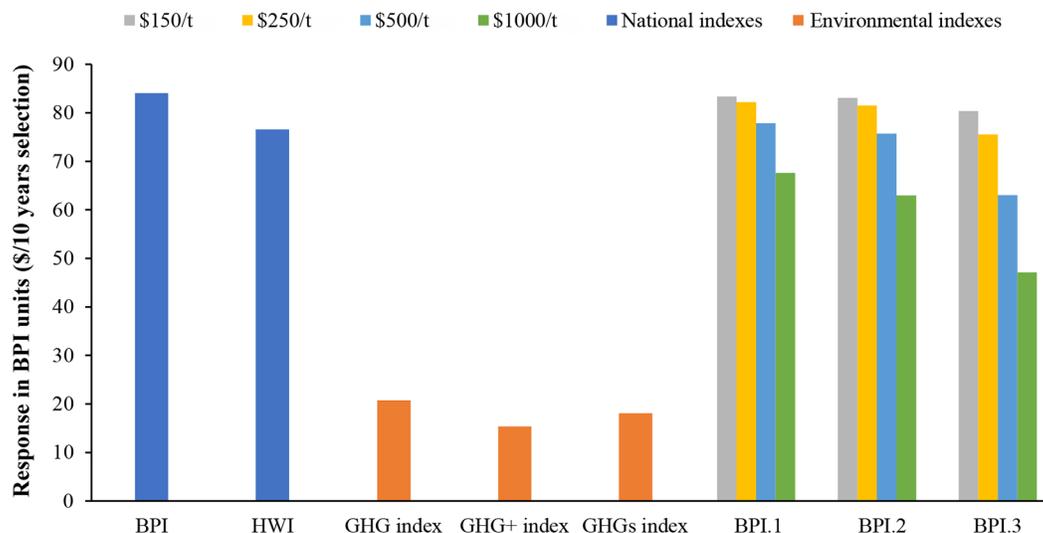
## Environmental Response

The expected reduction in emissions due to selection on each index with a selection intensity equivalent to 1 SD of BPI are presented in Figure 3, in kilograms of  $\text{CO}_2$ -eq. The environmental reduction estimated for selection on BPI and HWI was 19.45 kg of  $\text{CO}_2$ -eq and 42.80 kg of  $\text{CO}_2$ -eq per cow per BPI SD, respectively. The reductions as a result of selection for BPI.1 and BPI.2 were similar, with carbon prices of AUD\$150/t (29.28 kg of  $\text{CO}_2$ -eq and 29.93 kg of  $\text{CO}_2$ -eq, respectively), AUD\$250/t (35.08 kg of  $\text{CO}_2$ -eq and 37.82 kg of  $\text{CO}_2$ -eq), AUD\$500/t (46.86 kg of  $\text{CO}_2$ -eq and 37.82 kg of  $\text{CO}_2$ -eq), and AUD\$1,000/t (61.06 kg of  $\text{CO}_2$ -eq and 71.38 kg of  $\text{CO}_2$ -eq). The emissions reduction due to selection for BPI.3 was considerably higher at 92.69 kg of  $\text{CO}_2$ -eq, 117.25 kg of  $\text{CO}_2$ -eq, 152.20 kg of  $\text{CO}_2$ -eq, and 175.19 kg of  $\text{CO}_2$ -eq when the carbon price was AUD\$150/t, AUD\$250/t, AUD\$500/t, and AUD\$1,000/t, respectively. Selection solely on  $\text{GHG}_{\text{index}}$ ,  $\text{GHG}_{\text{index}}^+$ , and  $\text{GHG}_{\text{index}}^{\text{S}}$  resulted in emissions reductions per cow per BPI SD of 78.88 kg of  $\text{CO}_2$ -eq, 90.54 kg of  $\text{CO}_2$ -eq, and 188.51 kg of  $\text{CO}_2$ -eq, respectively.

The change in residual methane follows the same pattern, as presented in Figure 4, with selection on BPI resulting in the lowest reduction in a direct methane trait (0.75 kg of  $\text{CO}_2$ -eq) and selection for BPI.3 at an economic value of AUD\$1,000/t resulting in the largest reduction in residual methane (4.57 kg of  $\text{CO}_2$ -eq). For all carbon prices, the simulated residual methane trait with higher accuracy resulted in significantly larger reductions in methane emissions, whereas the reduction of direct methane via selection for the currently estimated residual methane trait was marginal.

## Opportunity Cost of Carbon to Farmers

For all scenarios investigated, the opportunity cost of carbon to farmers was far below the actual carbon price (Figure 5). The costs of carbon experienced by farmers were AUD\$73.25 and AUD\$120.28 for BPI.1, AUD\$93.51 and AUD\$139.36 for BPI.2, and AUD\$50.38 and AUD\$87.22 for BPI.3 at carbon prices of AUD\$150/t and AUD\$250/t, respectively. The ratio of the cost to the farmer relative to the carbon price became substantially smaller as the carbon price increased and when a higher-accuracy residual methane trait was included in the national index scenarios, such as BPI.3. At a carbon price of AUD\$500/t, the costs of carbon to the farmer were AUD\$226.92, AUD\$244.50, and \$58.42 for BPI.1, BPI.2, and BPI.3, respectively. When the carbon price was AUD\$1,000/t, the costs of carbon to the farmer were AUD\$395.10 for BPI.1



**Figure 2.** Economic responses to selection on each index for a 1-SD improvement in the Balanced Performance Index (BPI) or approximately 10 yr of selection, equivalent to AUD\$84.06, as BPI units are \$AUD per cow per year, at carbon prices of \$150/t (gray), \$250/t (yellow), \$500/t (light blue), and \$1,000/t (green). HWI = Health Weighted Index. The  $\text{GHG}_{\text{index}}$  was developed as described by Richardson et al. (2021c) and updated to include a gross emissions value for residual methane ( $\text{GHG}_{\text{index}}^+$ ) as well as a simulated residual methane trait with higher accuracy ( $\text{GHG}_{\text{index}}^S$ ). BPI.1, BPI.2, and BPI.3 describe scenarios where the  $\text{GHG}_{\text{index}}$ ,  $\text{GHG}_{\text{index}}^+$ , and  $\text{GHG}_{\text{index}}^S$  have been included in the BPI, respectively.

and AUD\$406.32 for BPI.2. The scenario that resulted in the most advantageous carbon cost for farmers was selection on BPI.3 at a carbon price of AUD\$1,000/t, where the opportunity cost of carbon to farmers was only 24% of the dictated carbon price at AUD\$237.25. Contrastingly, the cost of carbon for farmers when selecting for HWI was AUD\$320.77, meaning that the level of reduction achieved through selection on HWI is the equivalent of applying a carbon price of AUD\$320.77.

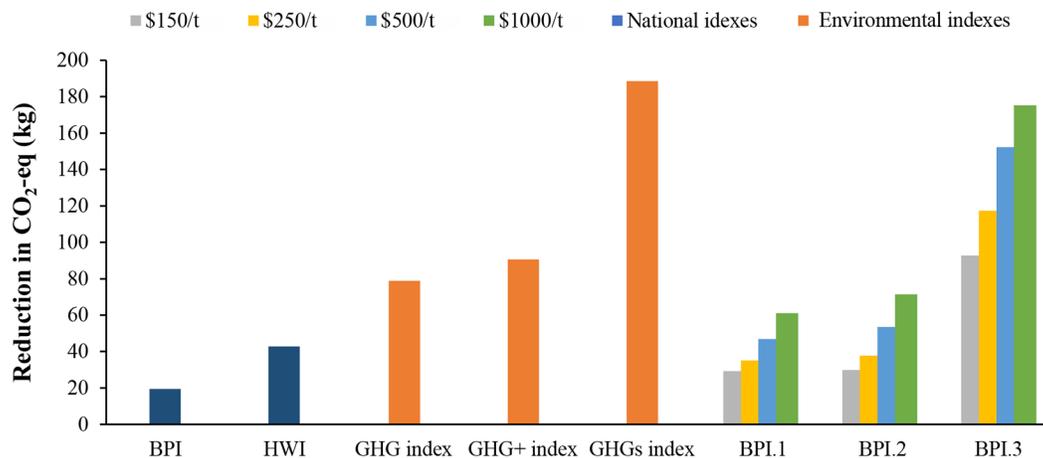
## DISCUSSION

The national index scenarios presented in this study represent viable possible options for current and future strategies to reduce gross emissions in Australian dairy cattle. The results indicate that incorporating an environmental index into the BPI would lead to a reduction in GHG emissions without a major reduction in profit, especially when an accurate residual methane trait is available.

There are currently 1.4 million dairy cows in Australia (Newton et al., 2020), and, at a national level, the inclusion of a GHG subindex into BPI would represent a substantial reduction in  $\text{CO}_2$ -eq. The average dairy cow in Australia produces roughly 4,298 kg of  $\text{CO}_2$ -eq and 58.06 kg of residual methane per year, based on the average residual methane produced per day (0.183 g of methane; Richardson et al. 2021b) and the  $c$  constant estimated in this study ( $c = 317d$ ). Although the

per-animal reduction in emissions appears small for the options tested that are available to implement immediately (29.28–71.38 kg of  $\text{CO}_2$ -eq and 0.88–1.22 kg of residual methane; Figures 3 and 4), the reduction has a substantial effect for the industry in terms of meeting reduction plans at the national herd level. A much larger per-animal reduction in emissions (92.69–175.19 kg of  $\text{CO}_2$ -eq and 2.19–4.57 kg of residual methane) may be achieved in the near future through selection on BPI.3, when a more accurate methane EBV becomes available. By implementing a GHG subindex in the national breeding program, we can achieve up to a 7.9% decrease in residual methane (Figure 5) and 9 times the reduction in gross emissions compared with the current breeding program, with little to no cost to farmers.

Assuming a per-cow current emissions output of 4,297.86 kg of  $\text{CO}_2$ -eq, an average production level of 339.29 kg of protein equivalents, a national herd size of 1.4 million cows, and current trait genetic trends (Richardson et al., 2021c), the reduction in gross emissions and emissions intensity that may be achieved through genetic selection was estimated for 10, 30, and 50 yr of genetic selection (Table 6). Using these parameters, gross emissions may be decreased by 2.78%, 8.23%, and 13.68% after 10, 30, and 50 yr of genetic selection for BPI.3 at a carbon price of AUD\$250/t; however, this translates to a cost to the farmer of AUD\$87.22/t. Interestingly, emissions intensity (**EI**) may be decreased by 7.84%, 21.25%, and 32.34% after 10, 30, and 50 yr of genetic selection for BPI.3 at the same carbon price,



**Figure 3.** Environmental response in kg of CO<sub>2</sub> equivalents (CO<sub>2</sub>-eq) per cow per year, based on selection on each index for a 1-SD improvement in the Balanced Performance Index (BPI) or approximately 10 yr of selection, equivalent to AUD\$84.06, as BPI units are \$AUD per cow per year, at carbon prices of \$150/t (gray), \$250/t (yellow), \$500/t (light blue), and \$1,000/t (green). HWI = Health Weighted Index. The GHG<sub>index</sub> was developed as described by Richardson et al. (2021c) and updated to include a gross emissions value for residual methane (GHG<sup>+</sup><sub>index</sub>) as well as a simulated residual methane trait with higher accuracy (GHG<sup>S</sup><sub>index</sub>). BPI.1, BPI.2, and BPI.3 describe scenarios where the GHG<sub>index</sub>, GHG<sup>+</sup><sub>index</sub>, and GHG<sup>S</sup><sub>index</sub> have been included in the BPI, respectively.

which is consistent with the 24% reduction in EI by 2050 reported by de Haas et al. (2021).

### Reduction in GHG

The largest reduction in emissions was observed when selection was based solely on ranking animals by the GHG subindexes and disregarding the current national breeding objective (Figure 3). However, a similar reduction in emissions was achieved when residual methane with an accuracy reasonable for implementation (0.54) was included in the index. For the versions of BPI tested, BPI.3, which included the GHG<sup>S</sup><sub>index</sub>, shows the largest reduction in kilograms of CO<sub>2</sub>-eq. This reduction was largely due to (1) the GV applied to residual methane and (2) including a higher-accuracy methane trait, causing residual methane to receive a considerable amount of relative emphasis within the GHG subindex. Residual methane has a global warming potential of 28, so whereas protein, for example, has a GV of 1.97, residual methane has a GV of 28. This causes a residual methane trait to be the largest driving force within the GHG<sup>S</sup><sub>index</sub>. Additionally, the higher accuracy allows for greater selection intensity to be applied, resulting in a greater response to selection in residual methane and subsequently in the GHG<sup>S</sup><sub>index</sub>.

### Short-Term Index Implementation to Reduce GHG

To the best of our knowledge, no residual methane EBV for livestock with an accuracy appropriate for industry implementation is available anywhere in the

world. Therefore, for the sake of a practical outcome, we will focus on considering the differences in environmental and economic changes between BPI.1 and BPI.2 for consideration as viable options for short-term implementation. The least computationally complex index is BPI.1, which includes the GHG subindex, composed entirely of currently estimated EBV. This index transitions relative emphasis from production and conformation traits onto traits related to survival, health, and efficiency by converting environmental coefficients into economic values. For implementation, this method takes advantage of the current genetic evaluation system and requires no additional EBV to be estimated. However, this method does not consider or fully capture the variation that exists in methane emissions between animals. As shown by our results, the current residual methane trait does not have much advantage in reducing emissions when compared with BPI.1, primarily due to its low reliability. A much larger reference population is required to reach the accuracy for implementation, particularly when such novel traits are developed using a female-driven reference population (Gonzalez-Recio et al., 2014). Although selecting for BPI.2 may not be substantially advantageous in terms of economic and environmental benefit, it does offer additional social benefits, such as maintaining the industry's social license to operate, by including the current residual methane trait at a lower accuracy. Therefore, until a sufficient amount of methane data is collected, BPI.1 is the most practical option in terms of economic and environmental benefit of the scenarios investigated to reduce emissions.

### Prediction Accuracy and Reference Population Size

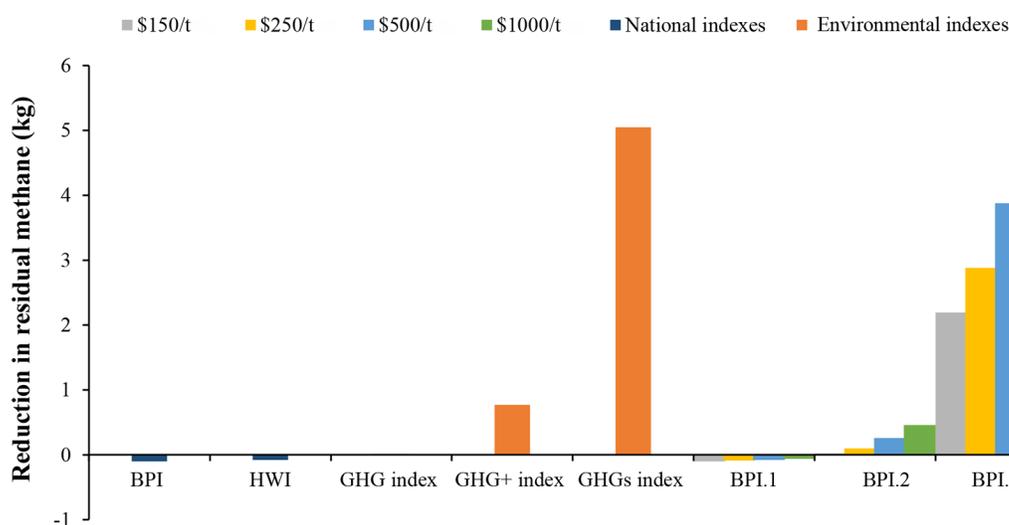
The goal of including a simulated trait within the GHG subindex was to demonstrate the level of emissions reduction that may be achieved by a trait with a reasonable accuracy for implementation in Australia, using a reference population size that may be reached in the near future. For estimating the accuracy of a methane trait in the future, we assumed that a reference population of 3,000 animals was reasonable; our assumption was based on comparable international collaborations used, for example, for genetic evaluations of feed saved (Bolormaa et al., 2021). Although pooling of international databases has proved to be more challenging due to the various trait definitions and apparatuses used for collecting raw data (de Haas et al., 2017), recent efforts have been successful in using an international multitrait genomic prediction for various definitions of methane (Manzanilla-Pech et al., 2021).

The challenge in implementing a GHG subindex that includes a novel trait, such as residual methane, is the building and maintenance of a reference population that is representative of the national population and sufficient to estimate accurate EBV. The feed saved and residual methane EBV are reliant to some extent on international data sharing (Pryce et al., 2015; Richardson et al., 2021b). Data-sharing risks and genotype  $\times$  environment interactions might mean it is prudent to focus data collection within country, although this is

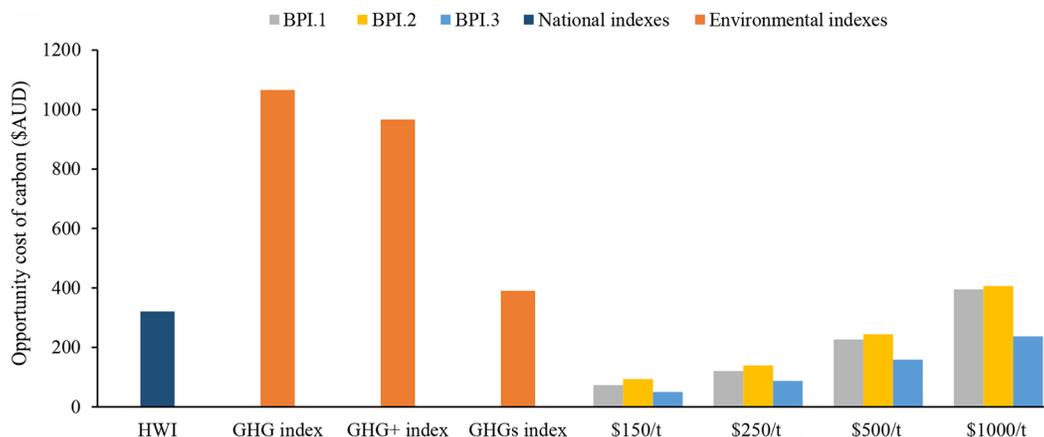
expensive unless easy-to-measure selection criteria can be found.

The method used in Australia to measure methane phenotypes ( $\text{SF}_6$ ; Deighton et al., 2014) is expensive, labor-intensive, and nearly impossible to implement on commercial farms. Another option is to include additional selection criteria, such as mid-infrared spectroscopy (Vanlierde et al., 2018), the heritable part of the microbiome (Zhang et al., 2020), feed efficiency, and phenotypically through volatile fatty acids (Williams et al., 2019) and rumen pH (Moate et al., 2020). If the required reference population size becomes available through proxy traits or international collaboration, it is likely that more records will be required to achieve the same level of accuracy, proportional to the genetic correlation, as often genetic correlations between countries (van den Berg et al., 2016; Haile-Mariam et al., 2020), measurement techniques and with proxy traits are less than one (Ismael et al., 2015).

There are several ways to calculate the prediction accuracy of larger reference populations. The assumptions behind these methods vary, which can result in considerable discrepancies between accuracies obtained (Brard and Ricard, 2015). Therefore, we also tested an approach applied by van den Berg et al. (2019), which uses the accuracy of cross-validation in a small data set to estimate  $\theta$  and apply the estimated  $\theta$  to a larger reference population size. This method indicated that approximately 15,000 animals may be needed to



**Figure 4.** Environmental response per cow per year based on selection on each index for a 1-SD improvement in the Balanced Performance Index (BPI) or approximately 10 yr of selection, equivalent to AUD\$84.06, as BPI units are \$AUD per cow per year, at carbon prices of \$150/t (gray), \$250/t (yellow), \$500/t (light blue), and \$1,000/t (green). Environmental response is presented for the residual methane trait (either currently available or simulated) for when each index is implemented. HWI = Health Weighted Index. The  $\text{GHG}_{\text{index}}^+$  was developed as described by Richardson et al. (2021c) and updated to include a gross emissions value for residual methane ( $\text{GHG}_{\text{index}}^+$ ) as well as a simulated residual methane trait with higher accuracy ( $\text{GHG}_{\text{index}}^{\text{S}}$ ). BPI.1, BPI.2, and BPI.3 describe scenarios where the  $\text{GHG}_{\text{index}}^+$ ,  $\text{GHG}_{\text{index}}^+$ , and  $\text{GHG}_{\text{index}}^{\text{S}}$  have been included in the BPI, respectively.



**Figure 5.** Opportunity costs of carbon emissions gains achieved through breeding for each index with emphasis on emissions were derived by taking the ratio of the reduction in Balanced Performance Index (BPI) units achieved in AUD\$ per cow per year relative to the reduction in CO<sub>2</sub> equivalents (CO<sub>2</sub>-eq) per cow per year when compared with the same index without any emphasis on emissions. In all scenarios, the opportunity cost of carbon to farmers was substantially lower than the applied carbon prices of \$150/t, \$250/t, \$500/t, and \$1,000/t. HWI = Health Weighted Index. The GHG<sub>index</sub> was developed as described by Richardson et al. (2021c) and updated to include a gross emissions value for residual methane (GHG<sup>+</sup><sub>index</sub>) as well as a simulated residual methane trait with higher accuracy (GHG<sup>S</sup><sub>index</sub>). BPI.1, BPI.2, and BPI.3 describe scenarios where the GHG<sub>index</sub>, GHG<sup>+</sup><sub>index</sub>, and GHG<sup>S</sup><sub>index</sub> have been included in the BPI, respectively.

achieve the desired accuracy of 0.5. Hence, a risk exists that that targeted reference population sizes may not deliver expected genomic prediction accuracies in the future, due to the uncertainty in predicting accuracies that may be obtained with future reference populations.

### Sacrifice in BPI

Overall, we observed that using the proposed indices (BPI.1., BPI.2, and BPI.3) would lead to a sizable reduction in GHG emissions without much compromise in genetic gain for BPI. This reduction in rate of genetic gain in BPI is driven primarily by a general decrease in selection responses for production and conformation traits, whereas greater gains are realized in feed effi-

ciency, fertility, health, and survival traits, as well as a large predicted responses in methane traits, especially when they are included in the subindex. The level of sacrifice in BPI progress is dependent on the economic value placed on carbon. In the scenario where carbon is given the economic value of AUD\$250/t, only 2 to 10% of BPI gains are to achieve 35 to 117 kg of CO<sub>2</sub>-eq reduction, depending on the GHG subindex included in BPI. However, given an economic value of AUD\$1,000/t of carbon, progress in BPI is still made, but at 70% of the progress compared with selection on BPI directly over a 10-yr period (Figure 2). The progress in BPI observed through selection on BPI.1, BPI.2, and BPI.3 at extreme values of carbon shows the contribution of survival, health, and efficiency traits within the BPI,

**Table 6.** Expected reduction in gross greenhouse gas (GHG) emissions and emissions intensity after 10, 30, and 50 yr of genetic selection on BPI.3<sup>1</sup> at a carbon price of AUD\$250/t or opportunity cost to farmers of AUD\$87.22/t

Period of genetic selection, yr <sup>2,3</sup>	Gross emissions <sup>4</sup>	Reduction in gross emissions, %	Emissions intensity <sup>1</sup>	Reduction in emissions intensity, %
10	5.85	2.78	11.68	7.84%
30	5.52	8.23	9.98	21.25%
50	5.20	13.68	8.57	32.34%

<sup>1</sup>BPI.3 describes a scenario in which the GHG<sup>S</sup><sub>index</sub> has been included in the Balanced Performance Index (BPI). The GHG<sub>index</sub> was developed as described by Richardson et al. (2021c) and updated to include a gross emissions value for residual methane (GHG<sup>+</sup><sub>index</sub>) or a simulated residual methane trait with higher accuracy (GHG<sup>S</sup><sub>index</sub>).

<sup>2</sup>Assuming a per-cow current emissions output of 4,297.86 kg of CO<sub>2</sub> equivalents (CO<sub>2</sub>-eq), an average production level of 339.29 kg of protein equivalents (protein-eq), a national herd size of 1.4 million cows, and current trait genetic trends (Richardson et al., 2021c).

<sup>3</sup>Protein-eq were estimated as described in Richardson et al. (2021c).

<sup>4</sup>Where gross emission is measured in million tonnes of CO<sub>2</sub>-eq and emission intensity is measured in kg of CO<sub>2</sub>-eq/kg of protein-eq.

as, in these scenarios, production and conformation traits receive negative weights. Interestingly, on average 18.06 units of BPI gain occur over 10 yr through selecting only on the GHG subindexes, which is 21% of the 10-yr gain in BPI if emissions were ignored.

### Comparing to HWI

When considering options to reduce emissions at the national level, the influence of current national indexes was also considered. In all scenarios, the reduction achieved by implementing a GHG subindex was greater than selection on BPI alone. However, at lower carbon prices, selection using the HWI resulted in a larger reduction in emissions compared with the indexes that did not include a reliable residual methane trait. In fact, the magnitude of the reduction in emissions from selection on HWI is comparable to BPI.1 at a carbon price of AUD\$500/t. However, by selecting on BPI.1, we reduce emissions by an additional ~4 kg of CO<sub>2</sub>-eq per cow per BPI SD while also achieving an additional 1.5 points of progress in BPI, compared with HWI. Therefore, the actual cost of carbon for farmers is only AUD\$226.92/t when selecting for BPI.1, whereas the cost of carbon for farmers is much higher at AUD\$320.77/t when selecting for HWI. This is due to the difference in relative emphasis for each trait within the respective indexes. Relative emphasis of traits within BPI.1 is more evenly distributed than HWI, with more weight on survival (16%), fertility (16%), and efficiency traits (11%). The HWI is dominated by fertility (29% relative emphasis), which leads to an expected comparable reduction in emissions as BPI.1. The BPI.1 at a carbon price of AUD\$500/t still maintains a reasonably high focus on production traits, with a total relative emphasis of 30%, compared with HWI where the relative emphasis drops to 23%. This comparison highlights the differences in the indexes that have similar reductions in emissions. The BPI.1 may be more appealing to farmers, as it still maintains a strong focus on economics, resulting in a similar emissions reduction with more emphasis on traits of high economic value. However, when the simulated residual methane trait is included, all of the current national indexes are substantially below the reduction achieved in emissions by selecting for BPI.3 at any economic value.

### Applied and Realized Economic Values of Environmental Traits

The reductions in methane are expressed in CO<sub>2</sub>-eq to allow for a more meaningful representation of the enteric methane reduction dealt with in this paper—

meaningful insofar as it allows for fair comparison to be made between sectors and gases. Additionally, it prepares the industry for the possible introduction of a carbon market, at which point a live economic value would need to be placed on methane.

Currently, there is no economic incentive for farmers to select for methane reduction. Unlike other traits included in the national breeding objective, no price signal currently exists to influence selection choices. However, it is expected that in the near future a carbon market will be implemented in Australia and could be introduced in agriculture sectors globally.

The price per tonne of carbon varies greatly by country and carbon market design. In Canada, this value is set to be approximately AUD\$180 (Government of Canada, 2021) in 2030, and in the United States and the EU the carbon price is currently set as AUD\$68 and AUD\$88/t, respectively (Stern and Stiglitz, 2021). These values are significantly lower than some of the economic values of carbon tested in the current study. The difference between the global economic values of carbon and the ones tested in this study reflects that (1) the carbon price is expected to increase substantially as national carbon markets continue to develop (Stern and Stiglitz, 2021); (2) some additional weight may be given to GHG subindexes to coincide with farmers' desires and achieve a level of emissions reduction deemed acceptable to maintain the industry's social license to operate (Martin-Collado et al., 2015); and (3) the realized cost to farmers is far lower than the weight placed on carbon due to simultaneous response in economically valuable traits.

The reduction in emissions achieved by including any of the GHG subindexes in the BPI is largely dependent on the economic value placed on carbon and the resulting percent emphasis the GHG subindex receives in the BPI. As the economic value of GHG increases, a point is reached where the national index would be selecting against protein, milk, and fat, as shown in the scenario where the carbon price is AUD\$1,000/t (i.e., the most valuable trait economically is methane). However, at a carbon price of \$1,000/t, the realized opportunity cost to farmers of including a GHG subindex in the BPI is substantially less than the carbon price (Figure 5). For example, at \$1,000/t carbon in BPI.1, the reduction in economic gain is \$16.44 (or 20% when GHG is not included in BPI). However, the benefit of this is an additional reduction of 41.61 kg of CO<sub>2</sub>-eq, resulting in a realized cost of carbon to producers of only \$395.09/t  $\left( \frac{-\$16.44}{-41.61 \text{ kg of CO}_2\text{-eq}} \times 1,000 \text{ kg/t} \right)$ . This is also the case at a carbon price of \$150/t, where the realized cost of carbon is \$73.24/t when selecting on BPI.1, and be-

comes even lower when selection is based on BPI.3 (\$50.38/t). When including environmental traits within an index, if a relatively low percentage of economic gain or index progression is sacrificed, then approximately 40 to 50% of the maximum possible reductions in emissions may be achieved. This principle is a major benefit of including a methane trait within the national index, as opposed to independent selection, as more progress is made by including all traits of environmental interest simultaneously, rather than selecting for the traits separately. This concurrent selection of EBV that have a correlated favorable response in emissions (BPI.1) in addition to direct selection on a residual methane trait (BPI.2 and BPI.3) allows a high level of methane reduction to be achieved with a realized cost to farmers that is far lower than the price of carbon.

### **Comparing Breeding Objectives for Gross Emissions and Emissions Intensity**

Previous studies have indicated 3 main opportunities to reduce emissions: (1) selection for a direct methane trait, (2) changes in herd structure, especially reducing replacements and therefore total emissions, and (3) increased production as means of diluting emissions per liter (Wall et al., 2010). Targeting options 1 and 2 is the focus of this paper, as they contribute to reducing gross emissions, which is consistent with the current national GHG reduction goals of Australia. The third option targets a reduction in EI through a dilution effect of generating more product per unit of emissions output.

This research focuses on reducing gross emissions, as this is the most likely breeding objective for the Australian dairy industry in the future. However, we do recognize that other breeding objectives that target a reduction in EI may be more favorable for some systems, especially in the agricultural sector. Although the current study only applied gross coefficients, methods exist that calculate coefficients based on intensity (Zhang et al., 2019) and have been estimated for the Australian industry (Richardson et al. 2021c).

The resulting difference when applying these intensity weights in the index is that increases in production traits are not penalized due to the dilution effect. An emission intensity reduction strategy is most effective in reducing a national industry inventory where production levels remain constant, as gross methane will also be reduced due to having fewer animals to produce the same level of output. However, reducing EI is not necessarily environmentally friendly if animal numbers remain the same or increase. In practice, at a policy level it is very hard to constrain the national level of output. The gross emissions approach used here is likely to be much more amenable to reducing Australia's

national inventory, but the global benefit of this would be diminished if loss of future increases in milk production from the Australian dairy industry result in higher future milk production from countries with a much less favorable EI than Australia (Ledgard et al., 2020).

### **Other Applications for GHG Subindex Values**

The implementation of each of the GHG subindexes will result in a reduction in emissions and increase in farm efficiency. This is done not only by selecting for a direct methane trait but also by quantifying the effects that other traits have on the level of methane produced. This is consistent with other studies, which have identified improvement in longevity as a major factor to reduce on-farm emissions when production or farm size are stationary (Lahart et al., 2021). The implementation of these indexes may help the industry reach state or national targets for emission reductions and international goals set for 2030 to maintain the 1.5°C warming rate.

The GHG indexes may also be used to quantify, on a large scale, individual animal GHG from a management perspective. As a management tool, the GHG subindex may be used to rank farms or individual cows and identify those animals or farms that are the lowest GHG emitters (Zhang et al., 2021). In terms of quantifying emissions inventories for individual farms or animals, base breed averages can be applied to translate genetic improvements back to the phenotypic scale as a way for farmers to document carbon release from cows. Finally, implementation demonstrates to the consumer that the dairy industry is dedicated to maintaining its industry social license to operate by reducing its emissions and maintaining its sustainable structure.

## **CONCLUSIONS**

The results presented in the current study indicate that, in the short term, a GHG subindex tool that farmers can use for industry application will be effective in reducing emissions while maintaining profits. Although the current estimated residual methane EBV is not accurate enough for implementation, GV coefficients may be applied to traits currently included in the national breeding objective to reduce emissions. Subsequently, when accurate residual methane EBV become available in the near future, a substantial increase in the reduction of emissions may be achieved, as shown through the simulated residual methane trait. The GHG subindexes in this study demonstrate the environmental benefit, with minimal economic sacrifice, of including a direct methane trait, as well as placing additional emphasis on traits known to affect emissions, in the

national selection index. By implementing a GHG sub-index in the national breeding program, we can achieve up to a 7.9% decrease in residual methane and 9 times the reduction in gross emissions in 10 yr compared with the current breeding program, with little to no cost to farmers. By 2050, selection based on the BPI.3 at carbon price of AUD\$250/t, or opportunity cost to farmers of \$87.22, will reduce gross emissions by 8.23% and EI by 21.25%.

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# **Chapter 6:**

## **Breeding for More Sustainable Dairy Cattle**

## **6.1 Breeding for more sustainable dairy cattle**

The traits and indexes in this thesis are a steppingstone towards long-term sustainability and cohesive breeding objectives for the Australian dairy industry. In agriculture, sustainability has a complex definition that includes increasing the production of a nutritionally dense, human-edible product to meet the pressure of a growing world population, while simultaneously reducing emissions, improving on farm efficiency, meeting social and societal expectations, and enhancing animal welfare. Additionally, these criteria must also consider the potential profitability of farms and overall maintenance of farmer wellness (Jones-Bitton et al., 2019). This robust definition of sustainability involves every aspect of the commodity chain and affects the entire dairy production system from the cow and the farmers to the consumer and the larger societal perception. Reducing methane and overall emissions is a component of the breeding objective required to create a sustainable dairy animal in the future. In this thesis I developed traits and built indexes to reduce emissions in dairy cattle, focusing on methane emissions. However, the methodologies and framework built through this thesis allow for future traits and criteria that require more complicated evaluations on their impact, and in return importance within our breeding program, to be simplistically implemented and included in the national breeding objective. This method can not only be applied in the dairy industry, but also in the larger, international livestock and agriculture sector to support more transparent comparisons between agriculture systems.

## **6.2 Review of important findings**

The results of Chapter 2 demonstrate the feasibility of defining and estimating a methane trait to reach the desired breeding objective effectively and efficiently, in this case, the reduction of gross emissions without affecting production. The first published paper of my thesis investigated possible methane trait definitions and successfully identified suitable candidate traits for inclusion in the national breeding objective. I

investigated nine candidate methane traits for inclusion in the Australian national breeding program that could reduce environmental impact without severely impacting other valuable traits such as production, health and fertility. The nine traits included residual methane phenotypically and/or genetically corrected for dry matter intake and/or energy corrected milk. Selection for a methane candidate trait also considered the viability of obtaining phenotypic measurements on a large number of individuals, as well as the trait's computational complexity and ease of implementation. Under these criteria, a methane production trait phenotypically corrected for energy corrected milk was proposed for inclusion in the national breeding program. This proposed residual methane trait was the most heritable of the residual methane traits ( $0.21 \pm 0.10$ ) and is appealing due to its strong statistical properties and ease of inclusion in a selection index. In a scenario where data is not limited, a residual methane trait where methane is corrected for intake level may also be a favorable option. However, methane and feed intake phenotypes were only available on a limited number of animals ( $n=464$ ), leading to residual methane estimated breeding values (EBV) with low reliabilities that were not yet suitable for industry implementation.

In Chapter 3, I explored options that did not require a direct methane trait, but rather relied on existing EBVs. Using methods developed by Amer et al. (2018), I calculated coefficients that describe the change in methane attributed to traits under selection in Australian dairy cattle in gross methane and methane intensity. Briefly, this method estimates the change in total emissions and product output caused by a one unit change in each index trait. This change is a result of either selection on a direct emissions trait (residual methane), changes in herd structure (fewer replacements), or the dilution effect of higher yields (milk production). The traits identified as having an unfavourable independent effect on gross emissions were milk volume, fat, protein, and fertility, whereas survival and feed saved had favourable effects on gross emissions. However, when considering emission intensity all traits had a favourable effect. Both coefficient definitions, gross or intensity, may be used in selection indexes to apply

emphasis on traits based on their environmental impact, depending on the desired breeding objective.

The final two chapters proposed greenhouse gas (GHG) sub-indexes and evaluated the implementation of these GHG sub-indexes in the Australia dairy industry on the basis of economic return and environmental reduction. In Chapter 4, we applied the estimated gross environmental coefficients estimated in Chapter 3 to calculate a GHG sub-index prototype, with the units of kg CO<sub>2</sub>-eq. The final set of EBVs selected for inclusion in the GHG sub-index were milk, fat and protein yield, survival, and feed saved as the effects of fertility are largely captured by the survival EBV. A further modification included a direct methane trait as defined in Chapter 2, residual methane phenotypically corrected for energy corrected milk, in the GHG sub-index. The accuracy of the GHG sub-index was 0.52 and calculated as the correlation between the index and gross methane, corrected for heritability ( $h^2 = 0.21$ ). The two GHG sub-indexes, with and without residual methane, were highly correlated, suggesting that the residual methane trait had a minimal impact on the GHG sub-index which was expected due to the low accuracy of the residual methane EBV.

Finally, Chapter 5 focused on developing options and strategies to implement a GHG sub-index in the Australian national breeding program. I investigated a total of 12 possible national selection index scenarios, by including three variations of a GHG sub-index at four economic weights within the BPI. The three developed GHG sub-indexes considered: 1) the application of gross environmental weights on previously derived EBVs (GHG<sub>index</sub>); 2) the possibility of including a direct residual methane trait as it is currently estimated into the previous GHG sub-index (GHG<sup>+</sup><sub>index</sub>) and 3) including a higher accuracy residual methane trait to demonstrate the impact of such a trait when one becomes available (GHG<sup>S</sup><sub>index</sub>). The results indicated that incorporating an environmental sub-index into the BPI causes a reduction in GHG emissions without compromising selection for profit to a great extent, especially when an accurate residual methane EBV becomes available. Although no residual methane trait with an accuracy

appropriate for industry implementation is available anywhere in the world, a national index which includes a GHG sub-index comprised of gross methane coefficients applied to currently evaluated EBVs, may be implemented in the short term as a tool that farmers can use for industry application to reduce emissions while maintaining profit, until a reliable residual methane trait is available. By implementing a GHG sub-index in the national breeding program, we can achieve up to a 7.9% decrease in residual methane and a 9 times greater reduction in emissions compared to the current breeding strategy, with little to no cost to farmers. By 2050, selection based on one of the more moderate index scenarios will reduce gross emissions by 8.23% and emissions intensity by 21.25%. Therefore, it is recommended that the Australian dairy industry offer a GHG sub-index as a mitigation strategy that will be effective at reducing emissions with little compromise to profit.

Chapter 5 draws together the main themes from my thesis and may be considered somewhat of a “pre-discussion” chapter. This final research chapter combines the results of the previous chapters to examine possible implementation strategies for the GHG sub-indexes and discusses the future implications of selection for a GHG sub-index in detail. In Chapter 5, I discuss the strongest driving forces behind reducing emissions, the challenges of implementing each of the proposed GHG sub-index, and approaches to develop and maintain a sufficient reference population for methane using proxy traits. The discussion of Chapter 5 considers the impact (environmental and economic) of selecting for a national index that includes a GHG component compared to current breeding strategies, and a detailed comparison to the HWI which could be considered the most sustainable-focused national index currently available in Australia. The potential value of methane in economic and social terms and the importance of maintaining the industry’s social license to operate were also discussed. Finally, in Chapter 5 I included a comparison of potential candidate methane traits for alternative breeding objectives, such as reducing emissions intensity, as well as other applications for the GHG sub-index outside the scope of genomic selection in herd management and emission inventory monitoring systems. Therefore, the following

general discussion sections are additional to those points that have already been discussed in the published work of Chapter 5.

### **6.3.1 Alternatives to incorporating a GHG subindex into the national breeding indices**

The results outlined in Chapter 5 clearly demonstrate the benefits of including a GHG sub-index in the national selection index to reduce the environmental footprint of the Australian dairy industry permanently and sustainably. However, in order to justify such a potentially significant change to an already well-established selection index (i.e. BPI), it is important to consider alternative approaches which may, on the surface, appear to offer quicker results and perhaps be simpler to implement. For example, I have been asked by both farmers and industry leaders - “Aren’t we working on a methane genomic estimated breeding value (**GEBV**)? Why not just release that and let market forces sort it out?”, often followed by “What if we just cull the top 10% highest emitters or don’t use the top 10% of high emitting bulls?”. These direct approaches therefore require:

1. The successful development of a methane trait or GHG subindex (Chapter 2 for details of GEBV) which is released separate from the national indices
2. Filtering on the basis of minimum “acceptable” criteria, otherwise known as independent culling or selection.

### **6.3.2 Stand alone ABV**

The feasibility of developing a methane GEBV was discussed in Chapter 2, and the merits of creating a GHG sub-index based on existing traits was discussed in Chapter 4. However, development is only part of the process, and it has been shown that the implementation method and engagement strategy are key factors in farmer

uptake and, subsequently, genetic progress. Introducing a GHG sub-index that is separate from a national selection index allows farmers to attend educational sessions and interact with the trait through presentations and user-friendly genetic evaluation interfaces. For example, by using the GoodBulls App (DataGene Ltd, 2017) which is used by Australian breeders to select bull teams through applying filters to bulls already awarded favourable bulls status on the basis of their BPI. The methane GEBV could be used as an additional selection criterion by the farmer without it contributing to the animal's national ranking. This is the case for the Heat Tolerance Australian Breeding Value (ABV; Nguyen et al.,2018) which remains a separate entity as the economic value of the trait varies greatly depending on region and exposure to heat stress. Similarly, the economic value for methane is currently unclear and the industry may also choose this option for methane until a mandatory carbon market is developed in Australia to dictate the carbon price for agriculture and livestock. Under this approach, farmers may use the separate value as a secondary selection criterion. For example, if a farmer is choosing between two high performance bulls, they may use the GHG sub-index and decide to use the bull that is higher performing coupled with lower emissions. However, selection indexes are the most efficient method to reach a breeding objective when multiple traits are involved. When traits are included in a selection index, we end up with a higher level of genetic improvement in all traits due to the favourable relationship traits share. This is shown in Chapter 5, where we can achieve 40-50% of the total reduction in emissions by sacrificing just a few points in BPI due to the favourable relationship between selection for the GHG index and traits of high economic importance such as health, fertility, and efficiency related traits.

### **6.3.3 Independent culling**

Independent culling is a selection strategy where an animal is removed from the herd if it does not meet specific requirements for a single trait (Hazel and Lush, 1942). At the farm level, this method may be used in cases where an animal has a production

level that is below a threshold that the farmer has predetermined as acceptable. Other applications include the eradication of diseases (e.g. Johne’s disease), health disorders (e.g. mastitis) and carrier status of lethal genetic defect (e.g. arthrogryposis multiplex congenita). Although this method leads to an immediate reduction in the population average for a specific trait, it does not change the direction or rate of genetic gain. Therefore, continual and periodic culling programs would need to be implemented to prevent the population from returning to its previous level, unless the trait under selection is eradicated (Scheper et al., 2016). In the context of what I am describing here, I will remove bulls that are: 1) among the highest 10% of emitters, or 2) positive on the GHG sub-index. Considering independent culling as a possible option to reduce emissions may be effective at reducing emissions in the short-term without significantly impacting BPI (Table 1); however, it is not a realistic long-term implementation strategy.

**Table 3:** Average index value for the top 300 and top 30 BPI bulls, when ranked by the three greenhouse gas (GHG) sub-indexes. In each group of bulls, two scenarios were explored. The first being if the top 10% of high emitting bulls were culled, and the second being if all bulls with appositve GHG sub-index value were culled.

	BPI <sup>1</sup>	GHG <sub>index</sub> <sup>2</sup>	BPI	GHG <sup>+</sup> <sub>index</sub>	BPI	GHG <sup>S</sup> <sub>index</sub>
Top 300 BPI bulls	228.75	62.67	228.75	53.10	228.75	71.81
cull top 10% emitters	228.80	49.81	228.62	37.51	228.67	38.25
cull all +ve GHG bulls	227.50	-40.37	228.82	-47.67	228.25	-109.24
Top 30 BPI bulls	311.98	67.29	311.98	51.39	311.98	72.16
cull top 10% emitters	313.43	53.99	314.11	36.11	313.83	40.71
cull all +ve GHG bulls	310.14	-28.34	313.79	-38.29	307.86	-110.72

<sup>1</sup>BPI is the Balance Performance Index

<sup>2</sup>GHG<sub>index</sub> applies the gross environmental weights to previously derived EBVs; GHG<sup>+</sup><sub>index</sub> includes the currently estimated direct residual methane trait in addition to the gross environmental weights, and GHG<sup>S</sup><sub>index</sub> includes a higher accuracy residual methane trait in addition to the gross environmental weights

Table 1 shows that independent culling may be an effective option to substantially reduce GHG emissions in the short-term for the bull population. In the case of bulls, the top 10% of high emitting bulls within the top 300 or top 30 BPI bulls may be culled without lowering the average BPI score. Even when all bulls with a positive GHG value are culled, the average BPI of the population is only slightly lowered while the average GHG index score is immensely reduced. This again highlights the findings for Chapter 5 which indicate that a large reduction in GHG emissions may be achieved with minimal impact on BPI. However, it is important to note that although the average BPI of the population remains practically the same between the independent culling scenarios and current national breeding program, the number of bulls dramatically decreases (Figure 1). Therefore, the potential impacts of such a selection strategy on future inbreeding levels, as well as the effects of decreasing the population size on genetic diversity and variation should be investigated. Although the reduction in GHG emissions by culling the top emitting bulls would trickle down to the cow level eventually, this selecting strategy would also be more challenging to



**Figure 1:** The GHG<sub>index</sub> value for the top 300 BPI bulls. An independent selection strategy would result in an immediate reduction in emissions with minimal loss in BPI if the top 10% of high emitting bulls (blue) or bulls with a positive GHG index value (orange) were culled. However, in the case of culling all positive GHG index value bulls, considerations should be made as the number of available bulls would be greatly reduced.

replicate at the cow level as it would result in a major decrease in the population size. Additionally, the reduction in GHG emissions achieved through independent selection may only be temporary. Although there would be an immediate drop in the average GHG emission of the dairy cattle population if the top 10% of high emitting animals were culled, the national herd would eventually return to the same level of emissions as the rate and direction of genetic gain were not influenced in the national breeding objective. As emissions cannot be eradicated, independent culling would simply result in a delay of negative environmental impacts rather than an improvement.

#### **6.4 The value of methane to maintain the industry's social license to operate**

Maintaining the industry's social license to operate is extremely important. While the results of Chapter 5 indicate that there is marginal economic or environmental impact when including the  $\text{GHG}^+_{\text{index}}$  sub-index in the national selection index, there are strong social reasons to do so. Introducing either  $\text{GHG}_{\text{index}}$  or  $\text{GHG}^+_{\text{index}}$  into the national index demonstrates to consumers that selecting for more sustainable animals is important to the dairy industry. Both of these sub-indexes account for the emissions generated by selecting for current index traits, however,  $\text{GHG}^+_{\text{index}}$  also includes a direct methane trait albeit with low accuracy. So, although both of the sub-indexes aim to reduce dairy cattle emissions,  $\text{GHG}^+_{\text{index}}$  has the added benefit of directly addressing the consumers concerns to reduce methane by including a direct residual methane trait (relative emphasis = 26%), while having marginal impact on the producer's profit and rate of genetic gain (less than \$0.50 over 10 years of selection compared to BPI.1; Chapter 5).

Eventually, as suggested in Chapter 5, a residual methane trait of higher accuracy will be developed to capture the genetic variation in methane emissions that exist between animals. This higher accuracy trait may be achieved by increasing the size of the reference population through a multifaceted approach which combines the use of proxy traits, international collaboration, and the collection of additional methane

phenotypes on Australian dairy cows. This dataset may also be expanded to include commercial animals through Ginfo herds, depending largely on funding opportunities and farmer willingness to participate in high labor data collection. Until this time, the residual methane trait as calculated in this thesis can act as an important place holder that allows farmers to become familiar and comfortable with a methane trait. This potentially gives us the best chance of higher acceptance and adoption rates by farmers in the long-term, as the more accurate residual methane trait will have a much more substantial relative emphasis (57%) within the GHG sub-index, as well as impact on BPI and national ranking.

### **6.5 The challenge of defining and estimating methane EBVs**

While the scope of this thesis is largely focused on the implementation of a residual methane trait, other methane traits have also been introduced and briefly discussed. These non-residual methane traits include methane production (kg/day), methane intensity (kg/ kg product output), and methane yield (kg methane/kg feed intake), and the benefits and drawbacks of each have been well summarized by de Haas et al (2017). Similar methods as applied to the residual methane traits in Chapters 2 to 5 may be used to estimate the emission mitigation potential achieved through genetic selection of these non-residual traits. However, small adjustments may be needed to avoid double counting and account for any unfavourable relationships that exist between methane and other index traits. For example, while selecting for methane production may be less computationally complex, methane production is strongly linked to milk yield and intake level. Therefore, applying strong selection pressure to decrease methane production would likely lead to lower producing cows. Alternatively, if we correct methane production for milk yield, such as in Chapter 2, we can simultaneously select for milk yield and residual methane. This is because residual traits are generally corrected for traits that are physiologically linked to avoid double counting, remove unwarranted genetic correlations, and accurately predict responses to

selection. This correction also ensures that when we are selecting for lower residual methane that we do not unintentionally lower production.

Currently, the size of national datasets for methane are too small for undergoing traditional routine genetic evaluations or accurately predicting EBVs, especially since the prediction equations are generally based on a female driven reference population from a few farms. González-Recio et al. (2020) suggested that for a moderately heritable trait ( $h^2=0.20$ ) measured in a female driven reference population, approximately 5000 individual animal records would be needed to reach a reliability high enough to be accepted for implementation ( $r^2=0.25$ ). Using the approach described by Goddard et al. (2011) and the specific residual methane trait definition applicable to the Australian dairy industry as described in Chapters 2 and 4, I estimated that by using 3000 animals an accuracy of 0.52 could be achieved. Although this offers insights into the range of population sizes we expect to require before an accurate trait implementation, it does introduce questions on the feasibility of reaching a reference population of this size.

Most studies have measured methane in lactating cattle. However, there is also an opportunity to measure methane in youngstock and in males, unlike many economically important dairy traits. Measuring methane in young bulls prior to puberty could aid in building reference populations, however, there are limitations to measuring methane in growing versus lactating animals. This has previously been observed in the development of feed efficiency genetic evaluations, where the genetic correlation between feed intake in growing heifers and lactating animals was 0.67 (Pryce et al., 2015) and has since lowered to 0.47 (Bolormaa et al., 2021). While obtaining records in youngstock and in males may increase the size of the reference population, it is vital that methane phenotypes be collected on lactating animals and that opportunities to build a female driven reference population continue to be explored.

International collaboration is underway to greatly increase the number of animals with recorded methane phenotypes in hopes to achieve the above estimated

level of accuracy. The methane dataset used in this study is part of the Efficient Dairy Genome Project, a large-scale international initiative aimed to increase feed efficiency and decrease methane emissions in dairy cattle through the utilization of genomics (Efficient Dairy Genome Project, 2016). This shared resource includes methane and dry matter intake data from six countries (Australian, Canada, United States, Switzerland, United Kingdom, and Denmark), sharing a total of over 3700 individual animals with dry matter intake records and almost 3000 animals with methane phenotypes (Manzanilla-Pech et al., 2021). From this dataset and other similar multi-national collaborations, substantial progress in dairy genetic research focused on efficiency has been achieved. As a result, feed efficiency traits which take advantage of the dry matter intake data available through these initiatives, are available in multiple countries including Australia (Pryce et al., 2015) and, more recently, the Netherlands (CRV, 2020), the United States (CDCB, 2020) and Canada (Lactanet Canada, 2021).

In 2015, through the success of international collaborations, Feed Saved was introduced to Australian farmers to improve on farm feed efficiency and sustainability (DataGene Ltd, 2020). Feed Saved is the Australian feed efficiency trait which consists of a lifetime residual feed intake (**RFI**) GEBV and bodyweight EBVs (Pryce et al., 2015). The lifetime RFI GEBV is made up of 2 components: RFI measured on cows and RFI measured on calves. Feed Saved is calculated by subtracting the animal's lifetime RFI from the feed required to maintain one kg of extra bodyweight and expressed so that a larger value represents a more efficient animals due to the lower maintenance costs of lesser-weight cows. When first introduced, the Feed Saved ABV was developed using a similar subset of animals as described in Chapter 2 (but with additional calf data, n=843 calf) and had a reliability of 0.50, with the RFI component having a reliability of 0.10 (Pryce et al., 2015). This genetic evaluation has since been updated to include the international cow data sources from the Efficient Dairy Genome Project and now the RFI life component has a reliability of 0.22 (Bolormaa et al., 2021). Therefore, it is reasonable to assume that a reference population of similar size,

achieved through incentivization of Ginfo herds and international collaboration, will be able to increase the reliability of a methane emission trait. However, the variety of measurement techniques and equipment used between countries may introduce complication when combining datasets as genetic correlations between countries are expected to be substantially less than 1 (van den Berg et al., 2016; Haile-Mariam et al., 2020).

Many different types of apparatus exist to measure methane emissions in dairy cows, including the gold standard respiration chambers (Moe and Tyrell et al., 1979), SF<sub>6</sub> technique (Deighton et al., 2014), sniffer method (Difford et al., 2018), and GreenFeed system (Hristov et al., 2016). Although concordance with the gold standard chambers were high for each method >0.7, the phenotypic correlation between measurement techniques was low to moderate (Garnsworthy et al., 2019). For example, the phenotypic correlation between SF<sub>6</sub> and GreenFeed was only 0.40; however, both methods were highly correlated to data collected using chambers (0.87 and 0.81 for SF<sub>6</sub> and GreenFeed, respectively). To overcome this challenge, an international consortium should be reached to develop standard operating procedures for collecting methane data when the intent is to share data internationally (e.g. which apparatuses to use, the time intervals to measure methane, and the other data and traits to record). This international procedure would limit the complications of combining datasets and offer insight into the most cohesive measuring techniques. However, even with standardized methane data collection procedures, an inexpensive proxy trait that can be, easily measure on-farm, may be needed to obtain enough data for accurate measures.

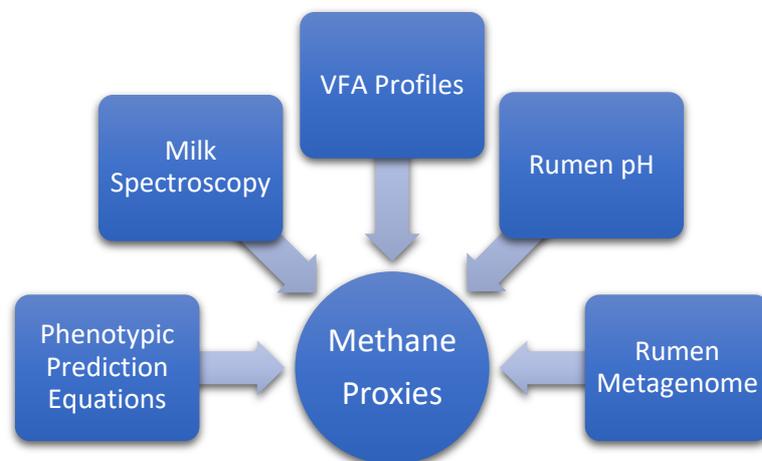
## **6.6 Developing a proxy trait**

Due to the expense and labour requirements of continually collecting methane data to maintain a reference population, methane predictor traits have also been explored. I have already briefly introduced some options which could be used to increase the number of phenotypes available for genomic predictions of methane.

Applying currently measured phenotypes, such as feed intake and production data, in mathematical prediction equations may provide insight into the level of methane production and efficiency of digestion. However, this method provides only an estimate of methane emissions and may not fully capture the variation in methane emissions that exists between animals.

MIR predicted methane phenotypes have proved challenging to implement in Australia ( $R^2= 0.30$ ) because of the five different feeding management systems used across the country. Some countries that have more homogenous feeding management practices, such as Belgium, have been more successful in using MIR to predict methane ( $R^2= 0.7$ ; Vanlierde et al., 2015). To the best of our knowledge, there are no studies currently available that have successfully used MIR predicted methane phenotypes to improve accuracies of genomic predictions of methane GEBVs. However, the application of MIR predicted phenotypes to improve the accuracy of genomic evaluations in Australia has been successfully achieved in other traits where the genetic correlation between the MIR predicted and measured phenotype is high (van den Berg et al., 2021). Luke et al. (2019) used blood urea nitrogen from research animals, in combination with phenotypes collected from commercial animals, to calibrate an MIR urea prediction equation that was then successfully applied to increase the accuracy of genomic prediction of urea (van den Berg et al., 2021). This method agrees with other studies showing that herd-by-herd validation methods are required to accurately extrapolate the MIR prediction to commercial animals (Wang et al., 2019). For methane, it may be challenging to obtain phenotypes on commercial animals, but these results indicate the possibility of improving genomic prediction through the application of quick and inexpensive proxy traits. However, before this method could be implemented in industry, further work is required to explore options to improve the prediction of methane using MIR in Australian dairy cattle, such as using multiple calibration equations for the different feeding systems and machine learning techniques.

The most promising proxy traits (Figure 2) include methane predicted using volatile fatty acid (VFA) profiles (Williams et al., 2019), rumen pH (Moate et al., 2020), and the microbiome (Wallace et al., 2015). Williams et al. (2019) demonstrated that VFA proportions in ruminal fluid can be used to predict methane yield and have suggested that this approach is amongst the cheapest methods for estimating methane yield of dairy cows. Rumen pH on the other hand has a strong biological connection to methane as the process of methanogens using excess hydrogen in the rumen to generate methane also drives feed fermentation by maintaining rumen pH (Moate et al., 2020). The rumen metagenome has also been shown to contain a heritable component, and Wallace et al. (2015) reported a significant difference in the abundance of microbial genes between high- and low-emitting beef steers. This proxy could be useful although difficult to scale up, but brings us closer to targeting the actual gut population producing methane. These methods are in the phenotypic development stage, and studies have yet to determine the genetic correlation between these proxy traits and direct methane traits, unlike other measured methane traits which have undergone genetic analysis. Therefore, future research should focus on obtaining a deeper understanding of methane and its biologically connected traits. This scope of research would encourage the discovery of better, more correlated proxy traits or the development of more accurate prediction equations using phenotypes which are easier and cheaper to measure, such as VFA and the rumen metagenome.



**Figure 2:** Potential proxy traits used to predict methane emissions

## **6.7 Introducing a carbon market and setting a carbon price in Australia**

There are large debates across Australian as to whether or not agriculture should be included in the emission caps program, and much of the discussion is based on taxing emissions (<https://www.abc.net.au/news/2011-07-15/carbon-tax-farmers/2795816>). Understandably, this has led to anxiety amongst farmers. However, there are other aspects and opportunities of a carbon market that have not been highlighted.

The introduction of a national carbon market would establish a structured carbon price in Australia and contribute to determining the emphasis a methane trait or GHG sub-index would receive in the national dairy breeding program, along with the social value of carbon. However, predicting the characteristics of a future carbon market is challenging. In Australia, and around the world, many aspects are still unknown regarding the introduction and adoption of a national carbon market. This uncertainty is even more so apparent in the development of an international market which would further escalate the value of carbon.

Currently, a carbon market does exist on a voluntary basis within Australia and has a carbon price of approximately \$20/tonne. Carbon markets are continuing to be developed internationally, although generally are country or region specific. The Canadian government has set a national carbon price of CAD\$170/tonne carbon (approximately AUD\$180) for 2030, which will be achieved by incrementally increasing the current carbon price of \$50/tonne over the next 10 years (Government of Canada, 2021). In the United States, the carbon market is state-specific with some states implementing a strong market, while others are veering away from carbon markets completely (Stiglitz and Stern, 2021). The global market price of carbon is continuing to fluctuate due to increasing global demand and mandatory government policies as more countries adopt carbon markets to meet the targeted emissions reduction of the Paris Agreement, with carbon prices ranging from \$0 to \$210. As carbon markets grow in popularity and expand internationally, it is expected that the carbon price will

drastically increase (Stiglitz and Stern, 2021). If a carbon tax or credit is placed on agriculture, this could be a major detriment or blessing.

The introduction of a carbon credit system would allow farmers to actively reduce their emissions, through genetic and management strategies, and sell their credits to higher emitting sectors to create an additional revenue stream. A credit also provides a financial incentive to help drive uptake of the  $\text{GHG}_{\text{index}}$  sub-index or increase buy-in to the national selection index. However, if a carbon tax system is introduced, a point may be reached where the carbon tax restricts the ability of even the most sustainable farms to be profitable. If farmers are taxed for emissions, there also needs to be some additional credit to recognize the role of dairy and other agriculture systems to sequester carbon. Whether a carbon tax or credit is introduced, this thesis gives a framework or tool that farmers can use monitor the emissions of individual animals on their farms.

In Chapter 5, I proposed that the  $\text{GHG}_{\text{index}}$  be included in the BPI at a carbon price of \$250/tonne. Although this value seems to be much larger than the carbon price suggested for Australia, there are practical reasons for this suggestion, the first of which being the response to selection. The  $\text{GHG}_{\text{index}}$  is expressed in kg of  $\text{CO}_2\text{-eq}$ , therefore, while \$250/tonne carbon seems expensive, this translates to an economic value of \$0.25/kg  $\text{CO}_2\text{-eq}$ . If the carbon price was lowered to the current carbon price (~\$20/tonne), the  $\text{GHG}_{\text{index}}$  would receive a relative emphasis less than 0.5% and there would be almost no response to selection as the economic value would have no to marginal value compared to other traits (\$0.02/kg  $\text{CO}_2\text{-eq}$ ). Secondly, the correlation between the current BPI index and BPI including the  $\text{GHG}_{\text{index}}$  at a carbon price of \$250/tonne was 0.98 (Chapter 5) and some re-ranking of bulls did occur. As the relative emphasis of the  $\text{GHG}_{\text{index}}$  within the BPI is marginal at the lower carbon prices, the correlation between the BPI and BPI.2 including the  $\text{GHG}_{\text{index}}$  at the lower carbon prices was >0.99. Therefore, suggesting little to no re-ranking or impact. Finally, in animal breeding, our breeding objective for current matings should be representative of the

animal we strive to produce in the next 10 years. If the average global carbon price is expected to increase substantially in coming years (Stiglitz and Stern, 2021), the economic value of the  $\text{GHG}_{\text{index}}$  should account for it. Although these carbon market scenarios are contrasting in terms of revenue stream the breeding objective is the same, to reduce emissions with increasing selection pressure as the carbon price increases. Therefore, it is recommended that a GHG sub-index be implemented into the national breeding program to capture potential benefit.

## **6.8 What if our breeding objective changes?**

The objective of this thesis was to examine the impact that animal breeding and genetics may have on developing a more sustainable dairy system by reducing gross methane emissions. The results of Chapter 5 indicate that by 2050 genetic selection alone could reduce the industry's gross methane emissions by 13.7%, if a  $\text{GHG}_{\text{index}}$  is included in the national breeding program at a conservative relative emphasis. Targeting gross emissions aligns with the Australian national inventory reports, is easily conceptualized by stakeholders, and computationally less complex in terms of generating EBVs (Chapter 2). However, it does not consider the growth required by industries, such as agriculture, which need to increase production to feed a growing global population. For example, it's very hard to simultaneously reduce gross emissions while also increasing milk production. Methane intensity is an alternative breeding objective that could be used to reduce emissions and target environmental productivity, by considering both the emissions and product output. This methane definition favours a more net neutral state and is especially efficient for systems where either animal inventories or production levels are capped, as both a reduction in emissions intensity and gross emissions may be achieved (Lahart et al., 2021). For example, Dairy Australia has set an industry goal of reducing its emission intensity by 30% by 2030, while the goal of DairyNZ is to reduce biogenic methane by 10% of 2017 levels by 2030 and 24 to 47% by 2050. Since 1990, the Australian dairy population has

decrease by 13% from 1.6 million to 1.4 million. Whereas in New Zealand, the dairy cattle population has increased by 82% since 1990 from 3.4 million to 6.3 million. Therefore, it makes sense the two countries may have different goals and strategy to reduce emissions. Due to the decreasing national herd size in Australia, both industry targets and national emissions inventory goals can be reached through selection on the  $\text{GHG}_{\text{index}}$ . In addition to the 13.7% reduction in gross emissions, the results of Chapter 5 indicated a simultaneous reduction of 32.3% in emissions intensity. This example helps to explain why the strategy for reducing emissions will be different for each country and highlights the complexity of the genetic component of a reduction strategy, such as choosing a breeding objective, in different industries.

While I have assumed farmers will be taxed or credited on the volume of emissions, it is possible that farmers will be taxed on a per animal basis as cows tend to be considered units by policy makers rather than animals with genetic variation. In this case, it may be more favourable for the breeding objective to target a reduction in emissions intensity to maximize the level of production for each cow. As shown through the intensity value coefficients estimated in Chapter 3, production and survival traits have the largest impact on emissions intensity and would therefore then become the target for genetic selection. Although a reduction in a direct emissions trait may be targeted, more genetic gain can be achieved in production, longevity and health traits and at a faster rate. The environmental and economic impact of adjusting the breeding objective to focus on reducing emissions intensity may be estimated by applying the intensity value coefficients using the same methodology as described in Chapters 4 and 5.

Another possible carbon tax or credit scenario is that farmers are penalized per hectare of land use. This is another definition of emissions intensity defined as emissions per hectare rather than production level. This scenario was investigated by Zhang et al. (2019), who again identified production, survival and fertility as traits with a prominent impact on reducing emissions. Traits of high economic importance being

identified as having favorable environmental impact is a reoccurring theme that has been reported by several research groups (Wall et al., 2010a; De Haas et al., 2021), and supports the concept that long-term sustainability may be achieved through economic and environmental security.

## **6.9 The future**

Based on the results of Chapter 5, the implementation of a GHG sub-index into the national breeding program may reduce gross emissions by 8.23% and emissions intensity by 21.25% by 2050. While this can be praised as an effective emissions reduction strategy with marginal cost to the farmer, it is important to note the structure of selection pressure within the dairy system and the delay of genetic progress in the female animals, which largely compose the national herd that is used to estimate emission inventories. Genetic progress in the dairy industry is driven by the paternal line due to the shortened generation interval and high selection intensity applied to artificial insemination bulls, compared to the on-farm maternal line (Shaeffer, 2006). However, the majority of dairy emissions are generated by the milking herd and replacement animals. Therefore, a low adoption rate of the GHG sub-index would result in a disconnect between the reduction in emissions achieved in high genetic performance animals and the reported emission inventories of the national herd. Therefore, high adoption rates are essential to align the reduction in emissions that may be achieved through genetic selection with the level of emissions reported in emissions inventories.

The tools developed in this thesis offer a foundation on which non-traditional breeding objectives can be considered and built based on a universal approach. From this work, future indexes that consider traits with non-economic value, such as environmental reduction or social purposes, may be developed. Urea and nitrogen production are traits of strong environmental interest for the dairy industry as nitrous oxide is another GHG frequently linked to agriculture and livestock (Gerber et al.,

2013). The viability of using quick and inexpensive methods to estimate an accurate urea trait introduces the opportunity for this trait to be included in genomic selection and NO<sub>2</sub> to be considered in future iteration of the GHG sub-index (Van den berg et al., 2021). The approaches used in this thesis can be used to model a range of GHG not just methane. For example, NO<sub>2</sub> is a GHG that is 298x more potent than CO<sub>2</sub>. While the biology of such traits is complex to model, coefficients can be estimated that describe the impact of current index traits on NO<sub>2</sub> emissions. Similar to the methane coefficient estimated in Chapter 3, these NO<sub>2</sub> coefficients could then be applied to existing traits and progress can be made to reduce NO<sub>2</sub> emissions until traits that target direct NO<sub>2</sub> emissions can be estimated. The two emission coefficients, methane and NO<sub>2</sub>, may then be combined to produce an aggregate GHG sub-index, expressed in kg CO<sub>2</sub>-eq, that more accurately estimate the GHG emission inventory of the Australian dairy industry by including multiple GHG profiles, as well as individual animal emission estimates that consider the genetic variation that exists within the dairy cattle population.

## **6.10 Concluding statement**

While the current Australian dairy industry is committed to sustainability through the Australian Dairy Sustainability Framework (2020), there is currently no focus on reducing emissions through the genetics of the national herd. The results outlined in this thesis clearly demonstrate the value of genetic selection to help the Australian dairy industry meet its sustainability goals. This thesis offers an applicable reduction program that may be easily and quickly implemented using genetic selection and the national selection indexes. Although my research has focused on reducing methane emissions in the productive herd and replacement animals, these methods may be extended to any GHG, animal class, or agricultural system to develop a more sustainable national breeding program.

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## **Appendix 1: Co-Author Contributions to Published Research Articles**

Maria Regina Caeli Richardson made significant leading contributions to all publications listed hereafter.

### **Chapter 2: Genetic parameters for methane emission traits in Australian dairy cows**

C. M. Richardson (CR), T. T. T. Nguyen (TN), M. Abdelsayed (MA), P. J. Moate (PM), S. R. O. Williams (SW), T. C. S. Chud (TC), F. S. Schenkel (FS), M.E. Goddard (MG), I. van den Berg (IV), B. G. Cocks (BC), L. C. Marett (LM), W. J. Wales (WW) and J. E. Pryce

Conceptualization: CR & JP

Sample acquisition: LM, SW, PM & WW

Methodology: CR, TN, MA & JP

Data analysis: CR, TN, TC & JP

Funding & resource acquisition: JP

Project administration: PM, WW & JP

Supervision: FS, BC, & JP

Writing & visualisation: CR & IV

Writing (review & editing): All authors

### **Chapter 3: Estimating methane coefficients to predict the environmental impact of traits in the Australian dairy breeding program**

C. M. Richardson (CR), P.R. Amer (PA), F.S. Hely (FH), I. van den Berg (IV), and J. E. Pryce (JP)

Conceptualization: CR, JP & PA

Methodology: CR, FH & PA

Data analysis: CR, FH & PA

Funding & resource acquisition: CR & JP

Project administration: JP

Supervision: JP & PA

Writing & visualisation: CR

Writing (review & editing): All authors

#### **Chapter 4: A method for implementing methane breeding values in Australian dairy cattle**

C.M. Richardson (CR), S. Bolormaa (SB), P.R. Amer (PA), I. van den Berg (IB), and J.E. Pryce (JP)

Conceptualization: CR, JP, & PA

Methodology: CR, PA & JP

Data analysis: CR, IV & BS

Funding & resource acquisition: CR & JP

Project administration: JP & CR

Supervision: JP & PA

Writing & visualisation: CR

Writing (review & editing): All authors

#### **Chapter 5: Reducing GHG emission through genetic selection in the Australian dairy industry**

C.M. Richardson (CR), P.R. Amer (PA), C. Quinton (CQ), J. Crowley (JC), F.S. Hely (FH), I. van den Berg (IV), and J.E. Pryce (JP)

Conceptualization: CR, JP & PA

Methodology: CR, JP, PA, CQ, JC & FH

Data analysis: CR, IV, CQ, JC & FH

Funding & resource acquisition: CR & JP

Project administration: JP & CR

Supervision: JP, PA, CQ & JC

Writing & visualisation: CR

Writing (review & editing): All authors

## **Appendix 2: List of Published Research Articles Contributed to During Candidature**

- Pryce J.E., **Richardson, C.M.**, Cheruiyot, E., van den Berg, I., Haile-Mariam, M., 2021. Using genetics to combat global warming and improve heat tolerance in dairy cows. *Submitted to IAEA International Symposium on Sustainable Animal Production and Health*
- Manzanilla-Pech, C.I.V., Gordo, D.M., Difford, G.F., Pryce, J.E., Schenkel, F., Wegmann, S., Miglior, F., Chud, T.C., Moate, P.J., Williams, S.R.O. and **Richardson, C.M.**, 2021. Breeding for reduced methane emission and feed-efficient Holstein cows: An international response. *Journal of Dairy Science*. 104: 8983-9001
- Malchiodi, F., Jamrozik, J., Christen, A.M., Fleming, A., Kistemaker, G.J., **Richardson, C.M.**, Daniel, V., Kelton, D.F., Schenkel, F.S. and Miglior, F., 2020. Symposium review: Multiple-trait single-step genomic evaluation for hoof health. *Journal of Dairy Science*. 103:5346-5353.
- Luke, T.D.W., Nguyen, T.T.T., Rochfort, S., Wales, W.J., **Richardson, C.M.**, Abdelsayed, M. and Pryce, J.E. 2019. Genomic prediction of serum biomarkers of health in early lactation. *Journal of Dairy Science*. 102:11142-11152.
- Martin, P., Baes, C., Houlihan, K., **Richardson, C.M.**, Jamrozik, J. and Miglior, F., 2019. Genetic correlations among selected traits in Canadian Holsteins. *Canadian Journal of Animal Science*. 99:693-704.

### **Appendix 3: List of Conference Presentations Completed During Candidature**

**Speaker:** A method for implementing methane breeding values in Australian dairy cattle. November 2021. 24<sup>th</sup> Conference of the Association for the Advancement of Animal Breeding & Genetics. Adelaide, Australia.

**Speaker:** Breeding for productive and sustainable dairy cattle. November 2020. 6<sup>th</sup> International Conference of Quantitative Genetics. Brisbane, Australia.

**Speaker:** Genetic parameters for multiple definitions of residual methane production in Australian dairy cattle. September 2019. International Symposium of Ruminant Production, Leipzig, Germany.

**Speaker:** Methane intensity and residual feed intake of lactating dairy cows. September 2019. International Symposium of Ruminant Production, Leipzig, Germany.

**Speaker:** Genetic parameters for environmental traits in Australian dairy cattle. August 2019. 70th Annual Meeting of European Federation of Animal Science. Ghent, Belgium.

**Speaker:** Searching for low methane animals. August 2019. 70th Annual Meeting of European Federation of Animal Science. Ghent, Belgium.

**Invited Speaker:** Using MIR for genomic predictions of environmental traits in dairy cattle. January 2019. Metabolomics meet Genomics in the Dairy Herd Improvement Symposium. Bundoora, Australia.

**Invited Speaker and Award Recipient:** Young Dairy Scientist of the Year: Breeding Burpless Bovines. February 2019. Australian Dairy Conference. Canberra, Australia.

**Invited Speaker:** Breeding for Efficiency: investigating dairy cattle efficiency traits and future implementation strategies. July 2018. American Society of Animal Science - Canadian Society of Animal Science. Vancouver, Canada.